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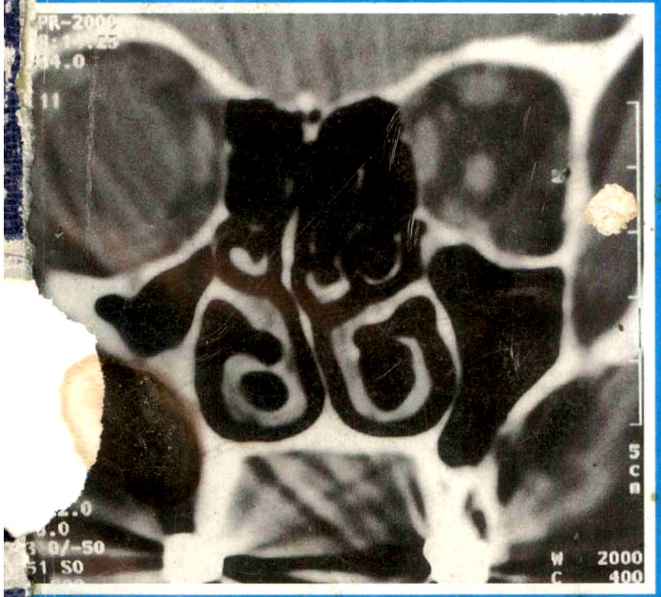

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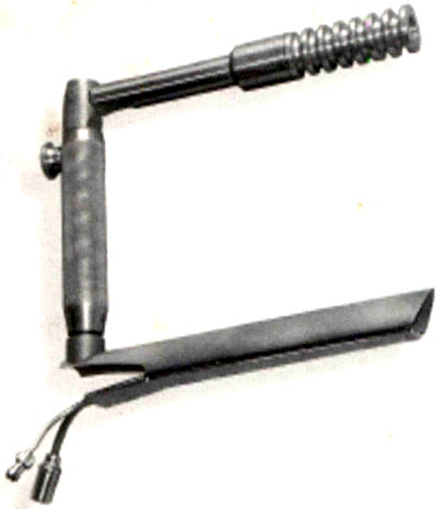

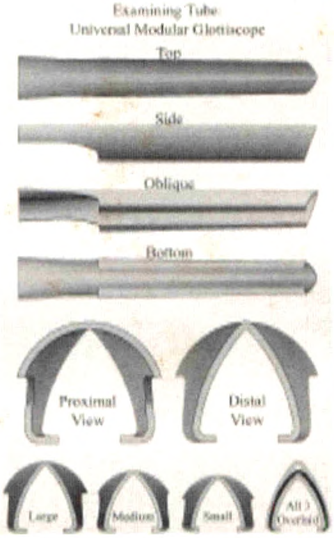

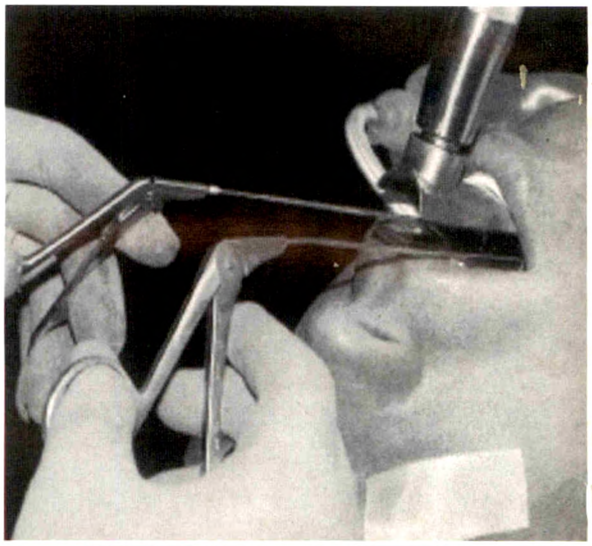

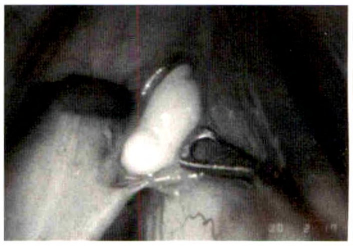

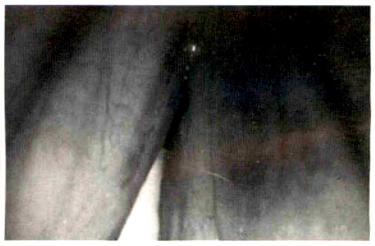

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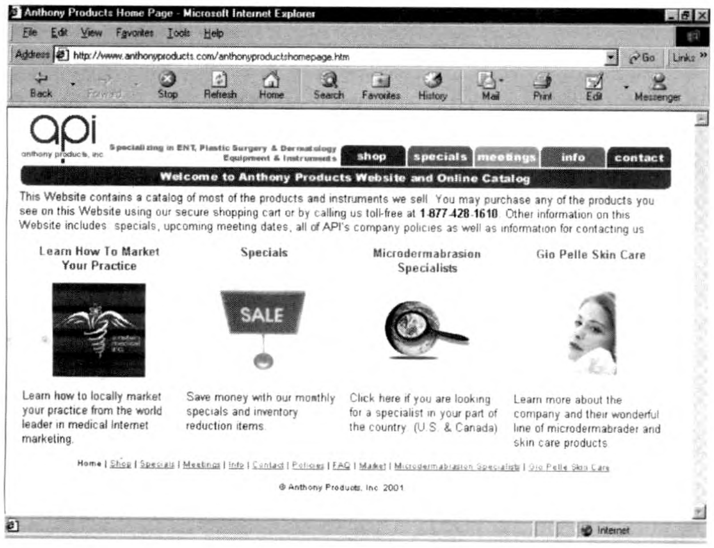
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LARYNX PRESERVATION WITH SUPRACRICOID PARTIAL LARYNGECTOMY WITH CRICOHYOIDOEPIGLOTTOPEXY

CORRELATION OF VIDEOSTROBOSCOPIC FINDINGS AND VOICE PARAMETERS

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Classically, the formation of a mucosal wave is dependent on the pliable mucosa present in the vocal fold. The supracricoid partial laryngectomy with cricohyoidoepiglottopexy is an organ preservation surgical technique in which both true vocal folds, both false vocal folds, both paraglottic spaces, and the entire thyroid cartilage are resected. The functional goal is speech and swallowing without a permanent tracheostomy. In an effort to further study voice production in these patients, we performed laryngeal stroboscopy in 5 patients. Analysis was performed with a modified Bless grading system. In addition, speech and voice parameters were also measured and correlated with stroboscopic findings. The key finding in this study was the presence of a periodic mucosal wave on the anterior aspect of the arytenoid cartilage, where it abuts the epiglottic cartilage. Patients with lower periodicity and symmetry scores tended to have lower jitter and shimmer percentages.

KEY WORDS — conservation laryngeal surgery, glottic carcinoma, laryngeal carcinoma, organ preservation surgery, supracricoid partial laryngectomy.

INTRODUCTION

The supracricoid partial laryngectomy (SCPL) procedures are organ preservation surgeries for the management of selected endolaryngeal carcinomas. The key to these procedures, first, is that the resection routinely includes removal of the entire thyroid cartilage, bilaterally, and the entirety of both true vocal folds and both false vocal folds. Second, it is important to understand that there are actually 2 types of SCPL that are performed for distinct indications.

In the version of the procedure that is used to manage selected supraglottic carcinomas, the entire epiglottis and preepiglottic space are resected and at least 1 arytenoid cartilage is spared. The reconstruction is accomplished by a pexis between the cricoid cartilage and the hyoid bone, hence the name supracricoid partial laryngectomy with cricohyoidopexy (SCPL-CHP).[1,2]

In the version of the technique that is used for selected glottic carcinomas, only the petiole and the inferior portion of the preepiglottic space are resected. After this type of resection, the cricoid cartilage is first sutured to the remaining portion of the epiglottis and then around the hyoid bone, hence the name supracricoid partial laryngectomy with cricohyoidoepiglottopexy (SCPL-CHEP).[2,3]

In general, there are 2 sets of oncological indications, which are 1) to be used to avoid total laryngectomy and 2) to be used in lieu of standard conservation laryngeal procedures in an effort to improve local control.[4] The specific oncological indications have been outlined in numerous publications.[5-9] The functional goals of both procedures are speech and swallowing without a permanent tracheostomy. The European series have achieved excellent functional outcome, and the procedures are presently being performed in the United States.[10]

Videolaryngoscopy is a useful adjunct for the diagnosis of disease within the larynx.[11] The multilayered structure of the vocal folds has been the basis for an anatomic understanding of the mucosal wave noted at the time of laryngeal stroboscopy.[12] The arytenoid mucosa has been noted to have a different histology, notably, mucoperichondrium that is densely adherent to the underlying cartilage.[13] In our efforts to understand the nature of the voice following the resection of both vocal folds during SCPL-CHEP, we performed laryngeal stroboscopy to discern the

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TABLE 1. CLINICAL FEATURES OF FIVE PATIENTS STUDIED WITH LARYNGEAL VIDEOSTROBOSCOPY AFTER SUPRACRICOID PARTIAL LARYNGECTOMY WITH CRICOHYOIDOEPIGLOTTOPEXY

Case	*Sex*	*Age (y)*	*T Stage*	*Prior Irradiation*	*Preoperative Vocal Fold Mobility*	*Preoperative Arytenoid Mobility*	*Arytenoid Resection*	*Date of Surgery*
1	M	35	r2	Yes	Normal	Normal	No	Feb 10, 1997
2	M	60	2	No	Normal	Normal	No	April 19, 1996
3	M	73	r2	Yes	Normal	Normal	No	May 24, 1996
4	M	55	3	No	Fixed	Impaired	Yes	Feb 17, 1997
5	F	68	r2	Yes	Normal	Normal	Yes	March 31, 1997

site of origin of the vibrating mucosa, which generates the vocal tones. Our focus in this study was on patients who had undergone SCPL-CHEP for selected glottic carcinomas. In a number of patients, after the SCPL, a periodic mucosal wave was noted at the level of the arytenoid cartilage.

In an effort to study mucosal waves that were present in areas other than the vocal fold, we developed a new classification schema for laryngeal stroboscopy following resection of various portions of the larynx. Other stroboscopic classification schemata focus on disease within an intact larynx; thus, their utility is limited in situations in which a part of the larynx has been surgically excised and reconstructed.[14] In this study, this new stroboscopic classification system was used to assess patients who underwent SCPL-CHEP. The characteristics of arytenoid mucosal waves observed with laryngeal stroboscopy were correlated with speech and voice parameters.

MATERIALS AND METHODS

Table 1 summarizes the clinical findings in 5 patients who underwent SCPL-CHEP at the University of Pennsylvania Medical Center. All patients were originally treated for squamous cell carcinoma of the glottis. All patients were alive and well without recurrence at the primary site after SCPL-CHEP. In addition, all of the patients underwent tracheal decannulation and were taking all nutrition by mouth without a gastrostomy tube. None of the patients had postoperative pneumonia, and all are swallowing without clinical evidence of aspiration. Four of the 5 patients returned to the occupations they performed before the surgery. The female patient was unemployed before the surgery and remained unemployed afterward. All patients underwent laryngeal videostroboscopy with the Kay RLS 9100 (Kay Elemetrics Corporation, Lincoln Park, New Jersey). All patients were followed a minimum of 6 months after the operation before undergoing stroboscopy.

A new modification of the University of Wisconsin videostroboscopic ratings system was utilized.[14] The observable characteristics of the vibratory pattern evaluated after SCPL-CHEP included symmetry (symmetric or asymmetric), periodicity (regular, irregular, or inconsistent), laryngeal closure (complete or incomplete), site of mucosal wave (anatomic location within the reconstructed larynx), type of procedure, mucosal appearance (dry, moist, edematous, normal), appearance of epiglottis (flat, crescent, omega, absent), and mucus (unremarkable, remarkable; see Figure).

Voice and speech parameters were analyzed with the Computerized Speech Lab and the multidimensional voice program from Kay Elemetrics Corporation. All patients' voices were recorded during the postoperative period under similar conditions, with a minimum of 6 months of follow-up before testing. Crevier-Buchman et al[15] have shown that stability of voice and speech parameters occurs at 6 months after SCPL-CHEP. The results are presented in Table 2.[16-19] The voice samples included maximum sustained phonation on the vowel "ah." Maximum phonation time (MPT) was defined as the best attempt to maximally prolong the vowel "ah" at a comfortable intensity. Average fundamental frequency (F0), jitter, and shimmer were automatically calculated from the number of glottal pulses per unit time.

RESULTS

Laryngeal Closure. The presence or absence of laryngeal closure following SCPL-CHEP was variable. Patient 2 had complete laryngeal closure, while patients 1, 3, 4, and 5 had incomplete laryngeal closure (Table 3). In general, laryngeal closure correlated with "breathiness" of voice quality.

Symmetry. Symmetry following SCPL-CHEP was partially affected by the resection itself. In cases in which 1 arytenoid cartilage was resected, asymmetry was always present to some degree. The asymmetry in patients in whom 1 arytenoid cartilage was resected was variable, because in some cases the spared arytenoid cartilage closed almost the entire laryngeal inlet, allowing symmetry between the vibrating surface of the arytenoid cartilage and the epiglottis anteriorly. In other cases, the spared arytenoid cartilage tended to localize to the ipsilateral side of the larynx, yielding more asymmetry. Nonetheless,

Subject______________Rater____________________ Date of Sample_______

LARYNGEAL CLOSURE Complete Incomplete Open Site(s) _______

SYMMETRY 1 2 3 4 5 6 7

Symmetrical Always Asymmetrical

PERIODICITY 1 2 3 4 5 6 7

Regular Always Irregular

SITE OF MUCOSAL WAVE____

Procedure Type:____

Mucosal Appearance: Dry Moist Edematous Normal

Epiglottis Shape: Flat Crescent Omega Absent

Mucus: Unremarkable Remarkable

__Anterior Mucus Pooling __Posterior Mucus Pooling

__Mucus Ball Formation __Mucus Stranding

Videostroboscopic ratings made after laryngeal resection.

variation was also noted among the patients in whom both arytenoid cartilages were spared. The range of symmetry ratings was 1 to 5, with an average of 2.40 (Table 3).

Periodicity. The ratings for periodicity were judged by the presence or absence of periodicity and the duration of formation of a periodic wave. The range of periodicity ratings was 1 to 7, and the average was 3.2 (Table 3).

Site of Mucosal Wave. The site of mucosal wave was observed between the anterior aspect of the body of 1 or both arytenoid cartilages and the tongue base or epiglottis in all cases. When both arytenoid cartilages had mucosal waves, the vibration was present both between the arytenoid cartilages and between the arytenoid cartilages and the rigid wall of the epiglottis anteriorly. When the site of vibration was only 1 arytenoid cartilage, the site of vibration was between the arytenoid cartilage and the epiglottis (Table 3).

Mucosal Appearance, Epiglottic Shape, and Mucus. The appearance of the mucosa was normal in all cases except case 5, in which mucosal edema was present. The epiglottis was noted to be flat in cases

TABLE 2. RESULTS OF VOICE ANALYSIS MADE AFTER SUPRACRICOID PARTIAL LARYNGECTOMY WITH CRICOHYOIDOEPIGLOTTOPEXY

Case	*1*	*2*	*3*	*4*	*5*	*Normal Adult Values*
/*a*/ MPT (s)	9 (↓)	28 (N)	6 (↓)	16 (N)	26 (↑)	12-28 (male)[16] 7-24.8 (female)[17]
/*a*/ AF (Hz)	118.9 (N)	169.4 (↑)	89 (↓)	124 (N)	91.8 (↓)	97-160 (male)[16] 175-192 (female)[18]
Jitter (%)	2.9 (N)	1.7 (N)	3 (↑)	10.8 (↑)	3.1 (↑)	0.2-2.9 (male)[16] 0.56-0.72 (female)[18]
Shimmer (%)	5.7 (N)	4.8 (N)	10.6 (↑)	36.3 (↑)	9.3 (↑)	0.5-8.6 (male)[16] 1.31-1.72 (female)[19]

N — normal; ↑ — increased; ↓ — decreased.

1, 3, 4, and 5, and a crescent-shaped epiglottis was noted in case 2. Mucus pooling was present in the 3 patients who underwent the procedure for irradiation salvage (cases 1, 3, and 5). In the 2 patients who were operated upon primarily, there were no abnormalities in the laryngeal mucus (Table 3).

Speech and Voice Parameters. Table 2 gives the acoustic parameters for each of the patients individually, as well as the norms for each parameter. The MPT was below normal in cases 1 and 3, within the normal range in cases 2 and 4, and above normal in case 5. The F0 fell into the normal range in cases 1 and 4, was elevated in case 2, and was decreased in cases 3 and 5. Jitter and shimmer were in the normal range in cases 1 and 2 and were elevated in cases 3, 4, and 5.

DISCUSSION

The quintessential function of the larynx is as a valve that sits atop the trachea, separating the airway from the alimentary tract. The fundamental functions of the larynx are in swallowing and respiration. In addition, normal laryngeal function allows for the generation of a tone for speech. Closure of the valve allows speech, swallowing, and behaviors such as lifting.[20] After SCPL, the remaining functional unit is the "cricoarytenoid unit," which consists of the arytenoid cartilage, the associated cricoarytenoid musculature, and the ipsilateral recurrent and superior laryngeal nerves.[21] Preservation of 1 "cricoarytenoid unit" with an appropriate reconstruction ultimately yields speech and swallowing without a permanent tracheostomy after SCPL.[22]

Voice is produced as a result of the interaction of 3 basic anatomic units. The infraglottic respiratory tract, which consists of the lungs, supporting muscles, back muscles, and diaphragm, with the support of the abdominal musculature, creates the power source. Second, the larynx serves as the oscillator. Third, in the normal larynx, the supraglottic vocal tract, including the supraglottic larynx, pharynx, and oral cavity, and the paranasal sinuses serve as resonators, although the infraglottic vocal tract may also have a resonating function.[23] After SCPL-CHEP, the resec-

TABLE 3. RESULTS OF VIDEOSTROBOSCOPIC ANALYSIS

Case	*Laryngeal Closure*	*Symmetry Rating*	*Periodicity Rating*	*Site of Mucosal Wave*	*Procedure Type*	*Mucosal Appearance*	*Mucus*	*Epiglottic Shape*
1	Incomplete, middle part of neolaryngeal inlet	1	1	Bilateral arytenoid	SCPL-CHEP	Normal	Remarkable for left anterior mucus pooling	Flat
2	Complete	1	1	Bilateral arytenoid	SCPL-CHEP	Normal	Unremarkable	Crescent
3	Incomplete, right part of neolaryngeal inlet	3	4	Left arytenoid	SCPL-CHEP	Normal, moist	Remarkable for right anterior mucus pooling	Flat
4	Incomplete, left part of neolaryngeal inlet	2	3	Right arytenoid	SCPL-CHEP with arytenoid resection	Normal	Unremarkable	Flat
5	Incomplete, right part of neolaryngeal inlet	5	7	Left arytenoid	SCPL-CHEP with arytenoid resection	Moist, edematous	Remarkable for posterior mucus pooling	Flat

tion includes the true and false vocal folds and the ventricles and saccules bilaterally. Hence, the mucosal structures normally involved in the "oscillator" functions of the larynx are no longer present after SCPL-CHEP. The "resonators," however, are essentially intact (except for the false vocal folds and ventricles) but repositioned. This study suggests that the basic "oscillator" remaining after SCPL-CHEP is the arytenoid mucosa.

The cover-body theory describes the vocal folds as a mucosal cover over the muscular body of the vocalis muscle. The stiff, underlying body in the intact larynx is responsible for the transverse movement of the vocal folds, whereas the looser mucosal cover vibrates primarily and, in forming a "fluid-like" traveling wave, generates sound.[24] Although the vocal fold mucosa appears to be the ideal structure for the creation of a periodic mucosal wave, experimental data have shown that a synchronous mucosal wave could be produced in a buccal mucosal flap that was used to replace the hemilarynx in mongrel dogs.[25] The mucosa overlying the arytenoid has been noted to be mucoperichondrium, which is densely adherent to the cartilage.[13] The presence of a periodic mucosal wave following SCPL-CHEP indicates that either a change has occurred in the arytenoid mucosa or else, perhaps, the essential mucosal layers needed to create a periodic mucosal wave are present in the arytenoid mucosa. Additionally, the anatomic position of the arytenoid cartilage has been changed after the operation, so that the anterior-superior aspect of the arytenoid cartilage abuts the epiglottis.

Interestingly, there are data to suggest that after resections of laryngeal tissue, the optimal voice outcome occurs when the reconstruction against which the normal vocal cord abuts is rigid, rather than being a mucosal surface with an asynchronous "mucosal wave."[26] Jiang and Titze[27] studied the vocal function of excised hemilarynges that abutted a vertical Plexiglas plate and concluded that near-normal phonation can result when 1 vocal fold vibrates against a rigid vertical wall. The findings in our study reveal that the mucosal wave occurs on the pliable mucosa of the arytenoid cartilage, including between the arytenoid cartilages, but primarily between the arytenoid cartilage and the rigid surface of the epiglottis. Andrews et al[25] studied a number of reconstructions following hemilaryngectomy in excised larynges of mongrel dogs. One of the reconstructions in their series included an epiglottic advancement similar to the Kambic reconstruction.[28] They noted that malpositioning of the epiglottic flap left a gap between the normal vocal fold and the reconstruction. In our series, 1 patient had a crescent-shaped epiglottis, and he tended to have a softer voice than the other patients, who had a flat epiglottis.

In normal speakers, increased vocal intensity correlates with greater amplitude of the mucosal wave.[11] Of interest, the speech-language pathologists participating in this study found that after SCPL-CHEP, when the intensity was either too low or too high, the periodicity of the mucosal wave became irregular. There is a clinical impression that there appears to be an optimal vocal intensity, unique to each patient, for yielding reproducible, regular periodicity. The clinical implication of this finding is that the speech-language pathologist may be able to help the patient attain improved vocal function by helping the patient identify his or her optimal vocal intensity. Future studies are warranted to compare changes in periodicity with variable vocal intensity.

The MPT has been reported to reflect the adequacy of air support during speech.[29] In other series, a decrease in MPT following SCPL-CHEP was explained by less laryngeal resistance with resultant loss of air during phonation.[16] Of note, in our series, the 2 patients (cases 1 and 3) who had a decreased MPT also had incomplete glottic closure, and the patient with the longest MPT had complete glottic closure. Nonetheless, the patients in cases 4 and 5 also had incomplete glottic closure, but had a normal MPT and an elevated MPT, respectively. These findings suggest that loss of air from an incompetent larynx may have played a role in MPT in some patients, but was not the only factor affecting MPT. Laccourreye et al[16] noted a strong statistical relationship between age and MPT following SCPL-CHEP, with a decrease in MPT with increasing age. Indeed, in our series, the 73-year-old patient (case 3) had the lowest MPT. One of the selection factors for SCPL-CHEP is clinical evidence of adequate pulmonary function, and all of our patients were able to climb 2 flights of stairs with no shortness of breath. It is likely that the excellent pulmonary function among the patients in this series diminished the impact of pulmonary function on the variability of MPT between patients.

In our series, the F0 was within the normal range in cases 1 and 4, decreased in cases 3 and 5, and increased in case 2. Our findings are different from those noted in a prior study in which the F0 in SCPL patients tended to be higher than that in normal subjects.[16] There does not appear to be any qualitative correlation between the findings on stroboscopy and the F0 in our patients.

Jitter and shimmer values allow for an analysis of the vibratory activity of the glottis.[30] Jitter is a reflection of the variability in F0, and shimmer reflects variability in intensity.[12] The patients in cases 1 and 2, both of whom had the best symmetry and periodicity values, had normal jitter and shimmer values. Conversely, the patients in cases 3, 4, and 5 had both asymmetry and irregular periodicity on stroboscopy, as well as jitter and shimmer values that were higher than normal. It is possible that the postoperative anatomic configuration in cases 1 and 2 yielded improved vibration characteristics of the arytenoid mucosa.

The literature suggests that SCPL-CHEP is quite valuable from the oncological perspective, and that swallowing and tracheal decannulation are generally successful.[1,3,31] What we have learned from performing SCPL-CHEP is that the vocal folds are not necessary for voice, per se, but they are necessary for high quality of voice. The voice is present after SCPL, but is generally quite hoarse.[16] Specifically, the roughness of the voice has been found to be significantly higher after SCPL-CHEP.[15] The postoperative voice is generally serviceable, as is evident in our patients, of whom 4 of the 5 returned to work at their original occupations, all of which require communication by speech among coworkers. (The female patient was unemployed before surgery.) Nonetheless, it is evident empirically that some patients have very good voices after SCPL-CHEP. Since hoarseness is one of the major issues after SCPL-CHEP, it is conceivable that qualitative improvements in the voice may have a major impact on the quality of life in these patients. Future studies should focus on identifying intraoperative maneuvers, as well as postoperative rehabilitation techniques, that may optimize the symmetry and periodicity of the arytenoid cartilage(s).

CONCLUSIONS

A mucosal wave is present on the anterior-superior surface of the arytenoid cartilages after SCPL-CHEP. When 1 arytenoid cartilage is present, the mucosal wave can be observed between the mucosa of the arytenoid cartilage and the epiglottis. When both arytenoid cartilages are present, the mucosal wave is noted both between the arytenoid cartilages and between the arytenoid cartilages and the epiglottis. Qualitative analysis revealed that patients with lower periodicity and symmetry scores tended to have lower jitter and shimmer percentages. Our modification of the Bless stroboscopic rating system was found to be a useful tool for assessment of laryngeal function following SCPL-CHEP.

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INSTABILITY OF EXTRACELLULAR MATRIX GENE EXPRESSION IN PRIMARY CELL CULTURE OF FIBROBLASTS FROM HUMAN VOCAL FOLD LAMINA PROPRIA AND TRACHEAL SCAR

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Primary fibroblast cell cultures were established from lamina propria of human vocal fold and tracheal scar. There exists a crucial need to provide new tools for studying voice biology, and one of the first steps is the development of a human primary laryngeal cell culture bank. Because cell lines can lose their differentiated phenotype in culture across passages, documentation of gene expression must be determined for passage populations, for us to have knowledge of cell behavior in vitro. Comparison of messenger RNA gene expression of extracellular matrix proteins (procollagen I, collagenase, elastin, hyaluronic acid synthase 2, hyaluronidase, fibronectin, cd44, fibromodulin, and decorin) across cell passages (3, 4, 5, 6, 10, and 12 for normal lamina propria and 3, 4, 5, 6, and 10 for tracheal scar) revealed varied growth patterns. Cytogenetic analysis demonstrated relative stability of the karyotypes across passages for the tracheal scar cell cultures, whereas the karyotypes of the normal lamina propria fibroblasts showed instability in in vitro cultures. Recommendations for use of primary cell cultures for further studies of gene expression are made.

KEY WORDS — cd44, cell culture, collagenase, decorin, elastin, fibromodulin, fibronectin, gene expression, hyaluronic acid synthase 2, hyaluronidase, karyotype, larynx, procollagen 1, vocal fold.

INTRODUCTION

Fibroblast cells play an important role in the regulation and maintenance of the extracellular matrix (ECM) of the vocal fold lamina propria.[1] Homeostatic regulation between the fibrous and interstitial proteins provides normal tissue structure and vibratory function. Previous research[2-6] has demonstrated that the quantity of lamina propria ECM proteins is altered in vocal fold disorders. The methods used, however, have been limited to histologic and immunohistologic techniques. In the literature, there is a paucity of work in which molecular biology methods were used to investigate gene expression and regulation, in normal or pathological vocal fold states. Unfortunately, studies of ECM gene expression and regulation that use these molecular methods have been hindered by the lack of availability of tissue samples. To assess molecular modifications at the cellular level, tissue samples must be received fresh within minutes of being isolated from the living body; otherwise, the RNA rapidly begins to degrade and is deemed unviable.

Primary cell culture is a technique that maximizes the use of a tissue sample, because it expands cell numbers by passing (or dividing) growing cells in vitro. In this manner, cells from one tissue sample can be multiplied and sequentially passed for use in numerous studies that examine various aspects of gene expression and regulation in vitro. To carry out such studies, however, and to expand cell numbers by passages in culture, it is important to have an accurate understanding of the behavior of the cells in the artificial environment. Furthermore, it is imperative to document the species verification with karyological analysis. Karyological analysis provides a means of monitoring changes in the chromosomal constitutions that may occur as passage numbers increase. A stable primary line will have no cross-contamination or chromosomal discrepancies.

To date, there have been no reports concerning long-term cultures of primary vocal fold lamina propria tissue. Uses of primary cell cultures represent a valuable tool in experimental lamina propria research for an improved understanding of normal and diseased tissue biology. We present the isolation, long-term culture, and characteristics of human vocal fold lamina propria and tracheal scar fibroblast cells. Culture fibroblasts from normal vocal fold lamina propria represent a normative control; tracheal scar fibroblasts will constitute a model of ECM scarring in

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TABLE 1. SEQUENCES OF POLYMERASE CHAIN REACTION PRIMERS, LENGTH OF PRODUCT, AND OPTIMAL CONDITIONS

	Primers (5'-3')					
	Forward	*Reverse*	*RNA (ng)*	*Product (bp)*	*Magnesium Chloride (mmol/L)*	*AT (°C)*
Elastin	AAAGCAGCAGCAAAGTTCGG	ACCTGGGACAACTGGAATCC	20	276	1.5	50
Procollagen 1	GTTGGTGCTAAGGGTGAAGC	ACCAGTGTCTCCTTTGCTGC	1.25	294	1.5	56
Collagenase (MMP-1)	CAACTTACATCGTGTTGCGG	ACCGGACTTCATCTCTGTCG	20	395	1.5	50
Fibronectin	TACCAACCTACGGATGACTCG	CATCATCGTAACACGTTGCC	1.25	300	1.5	56
Hyaluronic acid synthase 2	GTTGGCTACCAGTTTATCCAAACG	CTTTATGTGACTCATCTGTCTCAC	5	403	2	60
Hyaluronidase	TGGGGCTTCTACCTCTTTCC	TGAGGTACTGGCAGGTCTCC	2.5	421	1.5	62
cd44	GAAAGGAGCAGCACTTCAGG	CACTTGGCTTTCTGTCCTCC	20	397	1.5	50
Decorin	GATGCAGCTAGCCTGAAAGG	TCACACCCGAATAAGAAGCC	1.25	274	1.5	50
Fibromodulin	CACTTTCCTCCACAGATGCC	GCTGCTTTCTCAACTCAGCC	2.5	387	1.5	62
GAPDH	ACCCAGAAGACTGTGGATGG	TGAGCTTGACAAAGTGGTCG	Varied	382	1.5	56

AT — annealing temperature, GAPDH — glyceraldehyde-3-phosphate dehydrogenase.

the upper airway. Emphasis will be placed on ECM protein expression and genetic constitution in early and late passages. Genes of interest in normal vocal fold tissue include elastin, procollagen 1, fibronectin, hyaluronic acid synthase 2, hyaluronidase, cd44, and decorin. The aforementioned protein genes, in addition to collagenase (MMP-1) and fibromodulin, were studied in tracheal scar cell cultures.

METHODS

Isolation of Tissue Samples. Normal vocal fold lamina propria and tracheal scar tissue were obtained from a 78-year-old man and a 16-year-old boy who both underwent laryngeal surgery. Immediately after surgery, the tissue was placed in Eagle's minimum essential medium with Earle's balanced salt solution supplemented with 10% fetal bovine serum and 0.1 mmol/L nonessential amino acids (Sigma, Irvine, California; growth medium to be used throughout study). The University of Wisconsin–Madison Health Sciences Human Subjects and University of Utah Institutional Review Board committees approved the protocol for attainment of tissue samples.

Cell Culture. Tissue was rinsed in 75% ethanol, cut into small fragments under sterile conditions, and seeded on 60-mm cell culture dishes that were precoated with medium. Medium was added gently to the top of the tissue pieces. The medium was changed every 3 days for 14 days, after which fibroblasts had grown out from the tissues. The tissues were removed, and cells were grown to 80% confluence and then passaged to T75 flasks (passage 1). Passage treatment involved 0.125% trypsin treatment with division of the cells into 8 equal subsets. Six fractions were frozen in liquid nitrogen for long-term storage, 1 fraction was used for RNA extraction (harvested 5 days after split when 80% confluent), and 1 fraction was used for continued passage growth. These cells were plated and grown to 100% confluence, at which time they were passaged once more (passage 2, 1:8). Every 7 days thereafter, 100% confluent cells were trypsinized and passed (1:8), with the medium changed every second day to passage 10 for normal vocal fold and tracheal scar cells. The rate of fibroblast confluence for the normal lamina propria slowed after passage 10, with the fibroblasts taking 9 and 10 days to reach 100% confluence for passages 11 and 12, respectively.

Extraction of Total RNA. Total RNA extraction was completed on 1 fraction from each of passages 3, 4, 5, 6, and 10 for normal vocal fold lamina propria tissue and 1 fraction from each of passages 3, 4, 5, 6, 10, and 12 for tracheal scar tissue. Cells were lysed directly in flasks with Trizol reagent (Gibco-BRL, Life Technologies, Grand Island, New York). Total RNA was isolated via chloroform, precipitated via isopropanol, and washed with 75% ethanol. Total RNA was resuspended in diethyl pyrocarbonate–treated water, and the yield that was quantified by spectrophotometric analysis using 1 optic diameter at 260 nm equaled 40 µg of RNA per milliliter.

Reverse Transcriptase–Polymerase Chain Reaction Amplification. To eliminate genomic DNA contamination, we treated 1-µg aliquots of total RNA with 5 units of RNase-free DNase I (Amersham Pharmacia, Biotech, Piscataway, New Jersey) for 20 minutes at 37°C, followed by inactivation of the DNase I at 75°C. Reverse transcription was carried out for 90 minutes at 42°C in a volume of 20 µL containing 1 µg of DNase I-treated RNA, 20 mmol/L of deoxy-

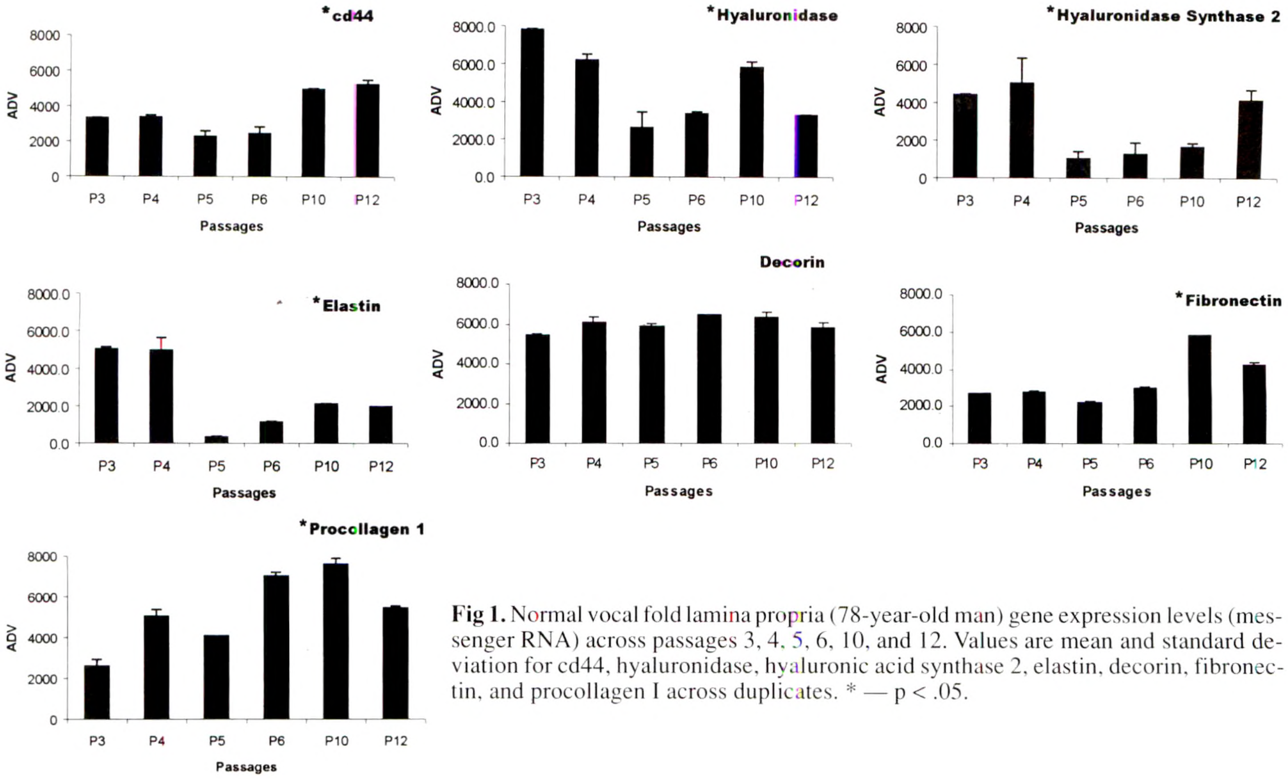

Fig 1. Normal vocal fold lamina propria (78-year-old man) gene expression levels (messenger RNA) across passages 3, 4, 5, 6, 10, and 12. Values are mean and standard deviation for cd44, hyaluronidase, hyaluronic acid synthase 2, elastin, decorin, fibronectin, and procollagen I across duplicates. * — $p < .05$.

nucleoside $5'$-triphosphates, 1.6 μg of Oligo-p(dT)_{15} primer, 50 units of Rnase inhibitor, 5 mmol/L of magnesium chloride, and 25 units of avian myeloblastosis virus reverse transcriptase in 1× reverse transcriptase buffer. Complementary DNA (cDNA) was stored in a volume of 100 μL at −80°C.

The oligonucleotide primer set sequences, the polymerase chain reaction (PCR) product sizes, and the optimal conditions of the primer sets used are listed in Table 1. The primer set sequences were designed from sequences published by GenBank Primer 3 (shareware program, National Institutes of Health, Primer3, 2000), with the exception of elastin.[7] DNA sequencing conducted at the University of Utah DNA sequence facility verified the sequences of the PCR products. The PCR reaction mixture consisted of (final volume of 25 μL) 10 mmol/L Tris–hydrochloric acid, 50 mmol/L potassium chloride, 1.5 to 2.0 mmol/L magnesium chloride, 0.2 mmol/L of each deoxynucleoside $5'$-triphosphates, 0.5 μM primers $5'$ and $3'$ respectively, 1 unit of Taq polymerase mixed with an equal volume of TaqStart antibody (Clontech Lab, Inc, Palo Alto, California), and various amounts of cDNA equivalent to 2.5 to 20 ng of total RNA. To avoid saturation caused by excess cDNA, we calculated the volume of cDNA used in each reaction through an optimization step. Simultaneous amplification (35 cycles) of twofold serial dilutions of the cDNA was completed, and the densities of the PCR products were plotted against the volumes of the starting cDNA. The cDNA amount chosen was based upon the PCR product that fell within a linear range before it reached maximum plateau. Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with all amplifications provided a comparative quantification of PCR products. All amplifications were completed in duplicate to verify validity and reliability. A Peltier Thermal Cycler PTC 2000 (MJ Research, Watertown, Massachusetts) was used. Amplification was carried out for 35 to 40 cycles. After an initial denaturation to 94°C for 3 minutes, each cycle consisted of denaturing at 94°C for 30 seconds, annealing at 50°C to 62°C (Table 1) for 30 seconds, and elongation at 72°C for 1 minute, except for the last extension, which lasted for 7 minutes.

A 2% agarose gel with 0.5 μg/mL of ethidium bromide was used for electrophoresis of the PCR products. Images were captured and analyzed with Gel-Expert (Nucleotech Corporation, San Mateo, California). To determine the level of transcript with standardized densitometry values, the ratio of target messenger RNA (mRNA) to the housekeeping gene GAPDH was ascertained.

Data from each protein gene expression level underwent 1-way analysis of variance across passages. Results were considered significant if the p value was less than .05.

Karyotype Analysis. Chromosomal analysis was performed on cells from 2 passages — 1 early passage (No. 4) and 1 late passage (No. 10) — for each

TABLE 2. ONE-WAY ANALYSIS OF VARIANCE SUMMARY TABLE FOR MEASURES RELATED TO MESSENGER RNA GENE EXPRESSION LEVELS IN NORMAL VOCAL FOLD LAMINA PROPRIA CELL CULTURE

Gene	*F Value*	*p*
Procollagen 1	145.8265	<.0001*
Elastin	98.4650	<.0001*
Hyaluronic acid synthase 2	16.3718	.0019*
Hyaluronidase	56.8158	.0001*
cd44	56.8021	.0001*
Decorin	3.8376	.0661
Fibronectin	522.4235	<.0001*

*p < .05.

sample to determine the presence of numerical and structural abnormalities. Specimens were processed by a routine procedure. Briefly, confluent cells were trypsinized and re-seeded in flasks and on coverslips. The cells were cultured in RPMI-1640 (Fisher Scientific, Houston, Texas) supplemented with 16% fetal bovine serum and Amnio Max supplemented medium (Life Technologies). Primary cultures were harvested after 2 to 4 days of growth. Air-dried chromosome preparations were GTG-banded, and karyotypes were described according to the ISCN (International System for Human Cytogenetic Nomenclature) 1995.[8]

RESULTS

Normal Vocal Fold Lamina Propria Cell Culture. The mRNA gene expression levels for procollagen I, elastin, hyaluronic acid synthase 2, hyaluronidase, cd44, decorin, and fibronectin in cell passages 3, 4, 5, 6, 10, and 12 are shown in Fig 1. Expression of each protein was evident at each passage, demonstrating retained expression throughout culture. Significant differences across passages ($p < .05$) were seen in all gene expression levels with the exception of decorin (Table 2), indicating considerable variability across passages.

Cytogenetic studies on the cells from passage 4 showed 4 different cell populations: 6 of 21 cells showed loss of the Y chromosome, 3 of 21 cells showed an apparently balanced translocation between chromosomes 13 and 14 in addition to loss of the Y chromosome, and 2 of 21 cells showed a male chromosome complement with multiple rearrangements involving chromosomes 2, 5, 10, 14, and 15. The remaining 10 of 21 cells showed a normal male chromosome complement. The karyotype was described as 45,X,–Y[cp6]/45,X,–Y,t(13;14)(q14;p11.1)[cp3]/46,XY,t(2;5)(q35;q15),t(10;14)(p15;q13),add(15)(q15)[cp2]/46,XY[cp10] (Fig 2). Evaluation of the cells from passage 10 showed a hypo-tetraploid chromosome complement with loss of a Y chromosome in all 20 cells analyzed. The karyotype was designated as 91, XXY,–Y.

Tracheal Scar Cell Culture. The mRNA gene expression levels for procollagen I, collagenase (MMP-

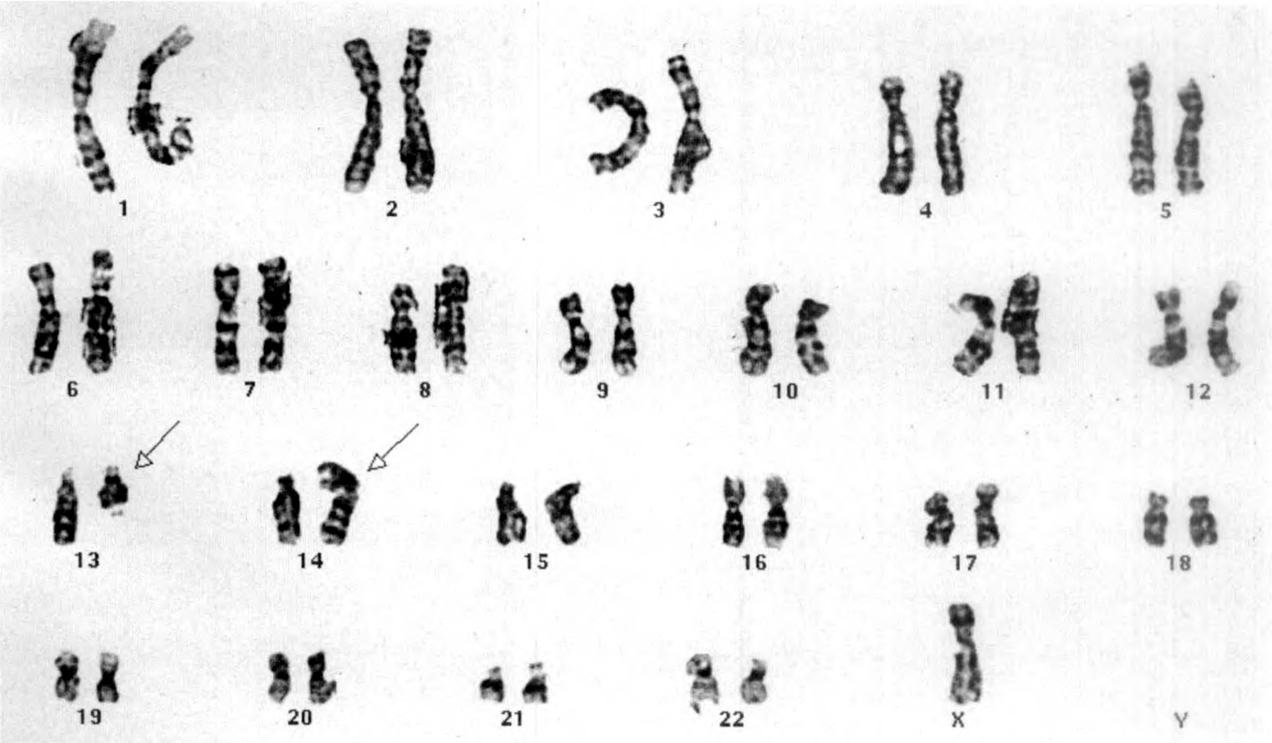

Fig 2. Cytogenetic studies on normal vocal fold lamina propria cells from passage 4 shows loss of Y chromosome and translocation between chromosomes 13 and 14, ie, 45,X,–Y,t(13;14)(q14;p11.1).

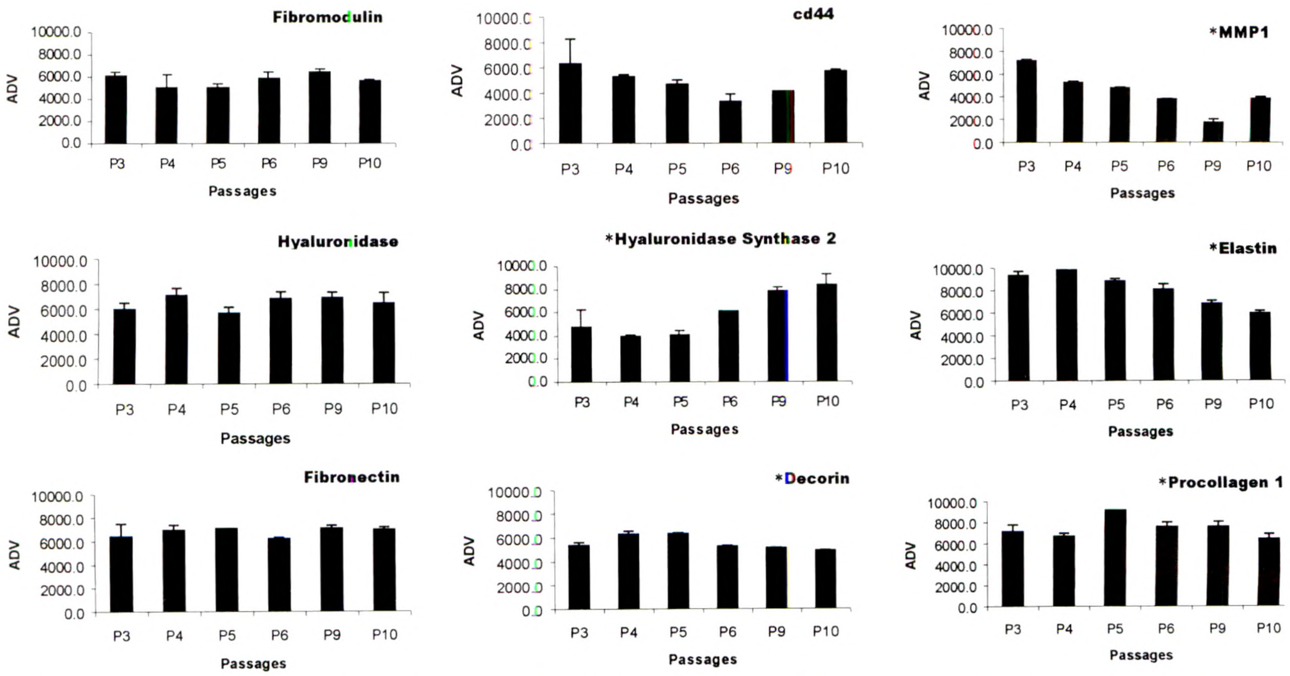

Fig 3. Tracheal scar (16-year-old boy) gene expression levels (messenger RNA) across passages 3, 4, 5, 6, 9, and 10. Values are mean and standard deviation for fibromodulin, cd44, MMP-1 (collagenase), hyaluronidase, hyaluronic acid synthase 2, elastin, fibronectin, decorin, and procollagen I across duplicates. * — p < .05.

1), elastin, hyaluronic acid synthase 2, hyaluronidase, cd44, decorin, fibromodulin, and fibronectin in cell passages 3, 4, 5, 6, 9, and 10 are shown in Fig 3. Retained expression throughout culture for each protein was established. Significant differences across passages (Table 3, p < .05) for procollagen I, elastin, hyaluronic acid synthase 2, decorin, and collagenase (MMP-1) indicate inconsistency through passages. Hyaluronidase, fibronectin, and fibromodulin expression levels did not differ across passage levels.

Metaphase cells examined from multiple cultures of the cells from passage 4 revealed a normal male karyotype in all 20 cells scored. Two cell populations were detected in the cells from passage 10. One cell line showed a tetraploid chromosome complement in 15 of 20 cells. The remaining 5 cells showed a normal male chromosome complement. The karyotype was designated as 92,XXYY[15]/46,XY[5] (Fig 4).

TABLE 3. ONE-WAY ANALYSIS OF VARIANCE SUMMARY TABLE FOR MEASURES RELATED TO MESSENGER RNA GENE EXPRESSION LEVELS IN TRACHEAL SCAR CELL CULTURE

Gene	*F Value*	*p*
Procollagen 1	10.1474	.0069*
Elastin	47.1559	.0001*
Hyaluronic acid synthase 2	14.3613	.0028*
Hyaluronidase	1.8279	.2417
cd44	3.0383	.1043
Decorin	35.0091	.0002*
Fibronectin	1.0908	.4508
Fibromodulin	2.1599	.1879
Collagenase (MMP-1)	274.3267	<.0001*

*p < .05.

DISCUSSION

The present study describes the isolation, culture, and characterization with genetic constitution, of fibroblasts from human vocal fold lamina propria and tracheal scar. The ECM is mainly composed of various proteins with their precursor, the fibroblast. Investigations into the regulation of the ECM in normal lamina propria and in disease states, ie, scarring, have been limited by the difficulty of obtaining fresh tissue specimens that can be used for molecular methods. Establishment of cell culture lines provides cells that can be used in a multiplicity of in vitro investigations.

Cells grown in culture have basic universal characteristics. One fundamental tenet is that there is a fixed limit to the number of divisions or passages that cells can undergo before they senesce — known as replicative senescence — and this limit is in some way related to aging of the organism.[9] In this investigation, normal vocal fold lamina propria fibroblasts were passed through passage 10, after which time, growth slowed (longer time to reach confluence). The tracheal fibroblast culture was passaged through to passage 10 without signs of slower growth. The normal vocal fold culture showed earlier signs of senescence than the scar culture, probably because the nor-

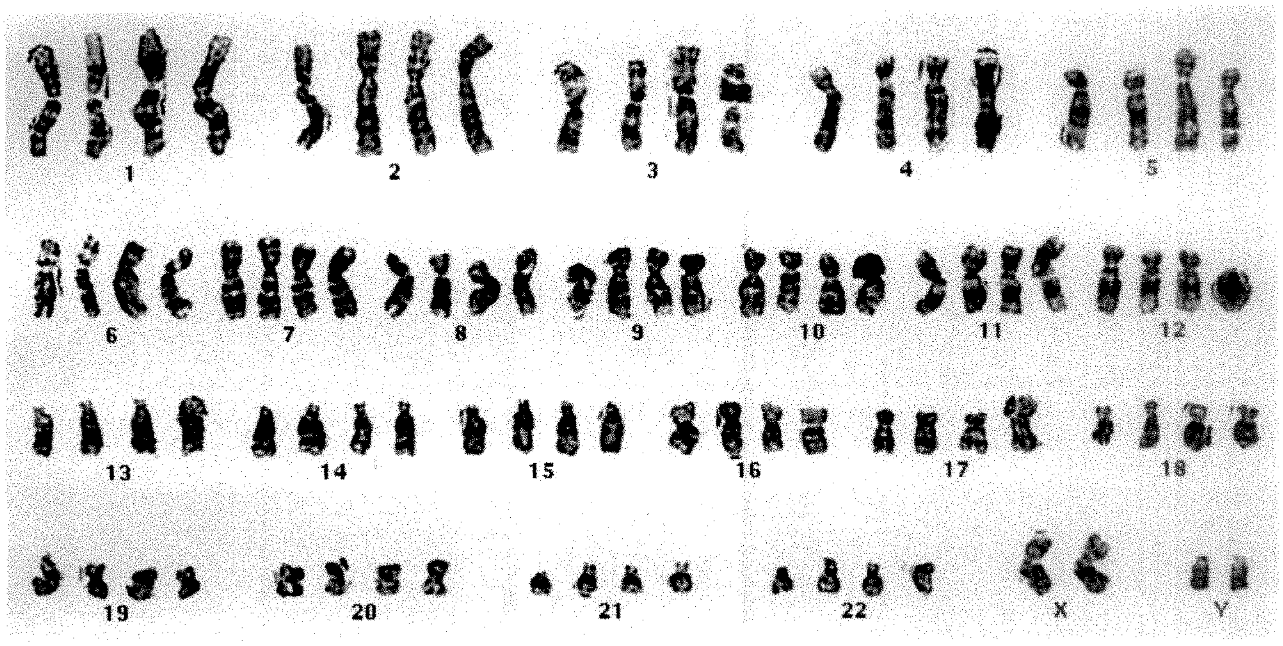

Fig 4. Chromosome analysis of tracheal scar cells from passage 10 shows tetraploid cell population, ie, 92,XXYY.

mal fibroblast culture was harvested from a 78-year-old, while the scar was taken from a 16-year-old. Laryngeal cells in culture may have differing cell division potentials, dependent upon in vivo aging; further investigation is warranted.

Analysis of ECM proteins was accomplished to determine their presence and stability across passages. Replicative senescent cells may continue to make proteins and express genes, similarly to presenescent cells[10]; however, these cells can show changes in cellular functions, hence altering their phenotypic expression.[11] Under in vitro conditions, proteins of interest were measured across all passages. Similar arbitrary dosimetry levels of gene expression were seen across cell lines, indicating equitable proliferation levels. Considerable variability was observed across passages within protein groups for each line. In normal lamina propria fibroblasts, all proteins, with the exception of decorin, demonstrated significant differences across passages. Tracheal scar fibroblasts demonstrated somewhat less variability, with hyaluronidase, cd44, fibronectin, and fibromodulin demonstrating nonsignificant differences across passages. Given that the cells were grown under consistent growth conditions throughout all passages, the differences in expression levels are not likely to have been due to the in vitro technique. Overall greater instability was demonstrated in the normal lamina propria fibroblasts, with altered gene expression phenotype, which is more than likely to be secondary to aging. The only discernible trend across all gene expression levels was a decrease at passage 5 for the normal fibroblasts. Because of the large amount of variability across passages with restricted trends, it would be prudent to use control cultures in experiments using laryngeal cell cultures.

Karyotype characterization across passages is necessary to substantiate the presence of contamination and alterations in chromosome structure. In this study, chromosome analysis of the normal lamina propria fibroblasts showed karyotypic instability across the cell passages. In passage 4, multiple chromosome abnormalities were observed. Loss of the Y chromosome, as the only change, has been well documented in hematopoietic cells in association with an aging phenomenon without clinical significance. However, loss of the Y chromosome as the primary change in other tissues has not been extensively reported in the literature. Therefore, the significance of the loss of the Y chromosome in the present study is unclear, although an aging-related phenomenon should be considered the most likely explanation. The presence of other atypical anomalies most likely represents in vitro genetic events as a result of long-term cell culture. The fact that chromosome analysis of the cells from passage 10 showed only a hypo-tetraploid karyotype without the presence of the abnormalities observed in passage 4 further indicates karyotypic instability of the normal lamina propria fibroblasts in in vitro cell cultures. On the other hand, cytogenetic analysis of the tracheal scar cells showed relatively stable karyotypes across passages without significant aberrations. The presence of normal tetraploid cells in passage 10 most likely reflects a culture artifact as a result of long-term in vitro culture. Because of the differences in the ages of the patients from whom the samples were taken, it appears as though the sample from the 76-year-old might be demonstrating a change in phenotype secondary to replicative senescence, while the sample from the 16-year-old was without phenotypic change at passage 10 — a finding that would correspond with the absence of senescence in growth for this line at passage 10.

CONCLUSIONS

The use of human primary laryngeal cell cultures will provide crucial resources for investigating regulation of vocal fold ECM in normal and diseased states. A thorough knowledge of the characteristics of each cell line used is necessary to draw valid and reliable conclusions. Because of the degree of instability described in gene expression between passages, use of multiple passages without controls in an experiment is not recommended. Karyotype characterization is a useful and crucial analysis to monitor alterations in the chromosome structure through cell growth and division. Replicative senescence appears to be a contributing factor in phenotypic expression of laryngeal cell lines from elderly donors, which have greater instability in the karyotype and gene expression, engendering caution with regard to their future use.

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AGE-RELATED CHANGES OF COLLAGENOUS FIBERS IN THE HUMAN VOCAL FOLD MUCOSA

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The viscoelastic properties of the vocal fold mucosa depend on the extracellular matrices. Collagenous fibers provide tensile strength and resilience and serve as a stabilizing scaffold in the extracellular matrices. Light and electron microscopic investigations of age-related changes of collagenous fibers in vocal fold mucosa were carried out on excised normal human adult larynges. Twelve human vocal fold mucosae were examined (10 from older adults with ages ranging from 70 to 97 years, and 2 from younger adults for comparison). The results for the aged mucosae can be summarized as follows. 1) Especially in men, collagenous fibers had increased and reticular fibers had decreased. 2) Collagenous fibers had formed bundles, and their density was high. 3) The spaces between collagenous fibers and the interstitial spaces for other extracellular matrices had decreased. 4) The collagenous fibril diameters differed, and their outline was irregular. 5) Twisted collagenous fibrils were present. 6) Occasionally, masses of dense collagenous fibers and fibrous tissue could be seen in the mucosa. 7) Occasionally, the collagenous fibers were increased all the way from the deep layer to the superficial layer of the mucosa, and consequently, there was no layered structure. Not only changes in the 3-dimensional structure of the collagenous fibers, but also their qualitative and quantitative changes, have an effect on the 3-dimensional structure of the extracellular matrices. Thus, the viscoelasticity must change to ensure normal phonation, and this change in viscoelasticity would explain one component of aging of the voice.

KEY WORDS — aging of vocal folds, aging of voice, collagenous fibers, extracellular matrix, larynx, ultrastructure, vocal fold.

INTRODUCTION

The viscoelastic properties of the lamina propria of the vocal fold mucosa are very important for the vibratory behavior of the structure. It greatly depends on the extracellular matrices.

Collagenous fibers provide tensile strength and resilience and serve as a stabilizing scaffold for the extracellular matrices. Reticular fibers are one of several molecular species of collagen. The reticular fibers and collagenous fibers have been found to be made up of different morphological forms of the same fibrous protein, collagen.[1] It is now generally accepted that the reticular fibers and collagenous fibers are essentially identical biochemically.[1] The size and pattern of the reticular fibers are different from those of the more typical collagenous fibers.[1] Our previous investigation revealed that the 3-dimensional (3-D) structure of the reticular fibers in the vocal fold mucosa is one of the key components of the structural maintenance and viscoelasticity of the vibrating tissue.[2] The complex of reticular fibers and other extracellular matrices is very important for the viscoelastic properties of the vocal fold mucosa.[2]

Age-related morphological changes in the reticular fibers and collagenous fibers influence the 3-D structure of the complex of reticular fibers, collagenous fibers, and other extracellular matrices; thus, they may influence the viscoelasticity of the vocal fold mucosa and become one component of vocal aging.

In this study, we used light and electron microscopy to focus on age-related changes in the collagenous fibers as an important component of the extracellular matrices in the human vocal fold mucosa.

MATERIALS AND METHODS

We used 10 normal aged human adult larynges obtained from autopsy cases with ages ranging from 70 to 97 years (5 men and 5 women) and 2 normal younger adult larynges from autopsy cases with ages of 33 and 34 years (1 man and 1 woman).

For light microscopic studies, the specimens were fixed in 10% formalin, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Hematoxylin and eosin, elastica–van Gieson, silver, and Alcian blue stains were used for each section. Elastica–van Gieson staining was used for the identification of collagenous and elastic fibers. Silver staining was used for reticular fibers and collagenous fibers, and Alcian blue staining (pH 2.5) was used for

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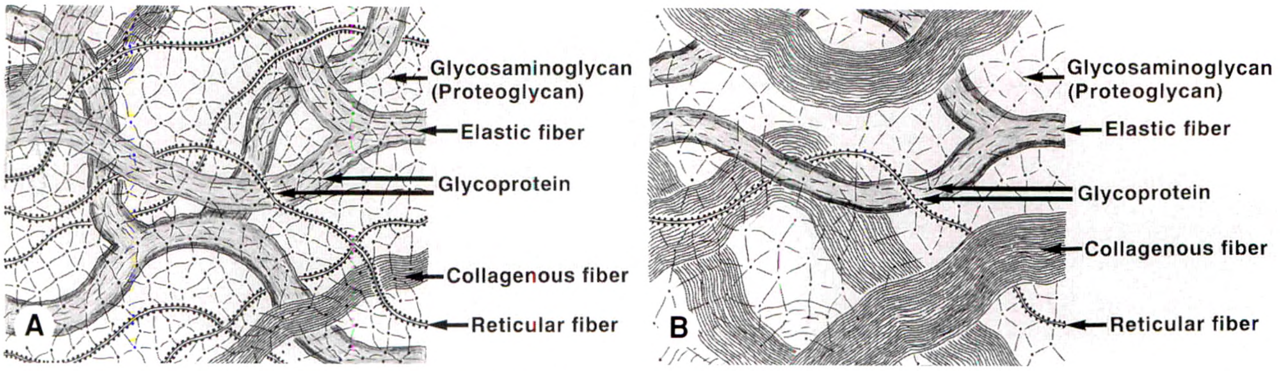

Fig 1. Schemata of extracellular matrices in superficial layer of lamina propria of human vocal fold mucosa. **A)** Younger adult. **B)** Aged adult.

glycosaminoglycan.

For transmission electron microscopy, the specimens were fixed in 2.5% glutaraldehyde at 4°C for 2 hours, rinsed with cacodylate buffer solution, and postfixed in 2% osmium tetroxide with cacodylate buffer solution at 4°C for 2 hours. After being rinsed with cacodylate buffer solution, the specimens were dehydrated in graded concentrations of ethanol and embedded in epoxy resin. Semithin sections were prepared with an ultramicrotome, stained with 1% toluidine blue, and examined with a light microscope. Thin sections were made with an ultramicrotome and stained with uranyl acetate–lead citrate and with tannic acid (to reveal glycoprotein and elastin). Observations were conducted with a JEM-2000EX transmission electron microscope (Japan Electron Optic Laboratory Co Ltd, Tokyo, Japan).

RESULTS

Superficial Layer of Lamina Propria of Younger Adult Vocal Fold Mucosa. Figure 1A shows a schema of the collagenous fibers, reticular fibers, and other extracellular matrices in the superficial layer of the lamina propria of a younger adult vocal fold mucosa.

In the younger adults' vocal fold mucosa, collagenous fibers were sparse in the superficial layer of the lamina propria (Figs 1A and 2A). The collagenous fibers consisted of numerous collagenous fibrils that were approximately 50 nm in diameter. Reticular fibers, one of several molecular species of collagen, were found in the superficial layer of the lamina propria of the vocal fold mucosa. The reticular fibers were made up of unit fibrils (Fig 2B) that were about 40 nm in diameter and had cross-bands with a periodicity of about 67 nm. The slender fibrils were wavy and did not form bundles. They formed delicate 3-D networks.

The spaces among the reticular and collagenous fibers for the other extracellular matrices were relatively large in the superficial layer of the lamina propria of the vocal fold mucosa (Figs 1A and 2). The other extracellular matrices, such as glycosaminoglycans, elastic fibers, and glycoproteins, were distributed in the spaces among the reticular and collagenous fibers.

Intermediate and Deep Layers of Lamina Propria of Younger Adult Vocal Fold Mucosa. In the younger adults' vocal fold mucosa, collagenous fibers were

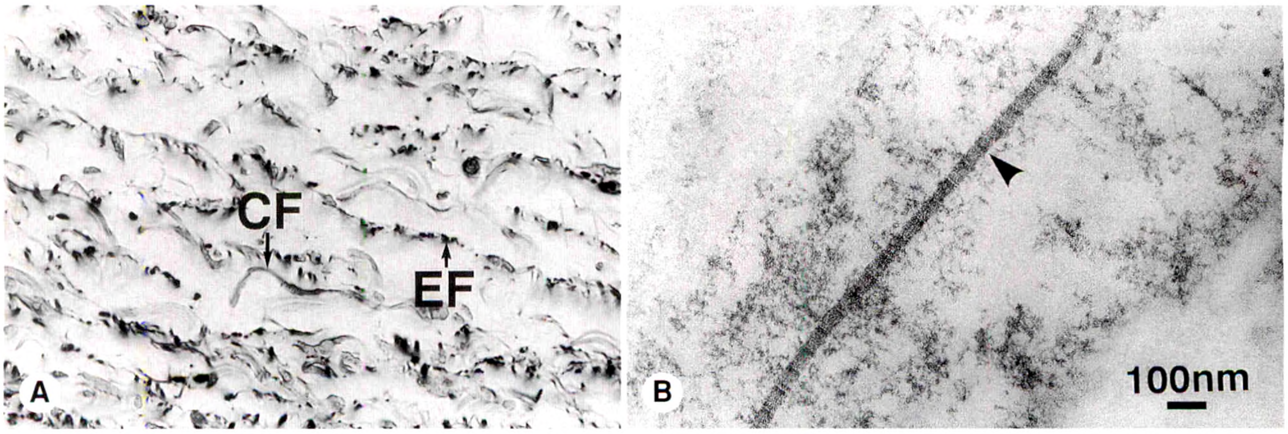

Fig 2. Laminae propriae of younger adults. **A)** Light microscopic view of superficial layer (33-year-old woman, elastica–van Gieson stain, original ×400). CF — collagenous fibers, EF — elastic fibers. **B)** Transmission electron microscopic view of superficial layer (34-year-old man, uranyl acetate and lead citrate stain). Arrowhead — reticular fiber.

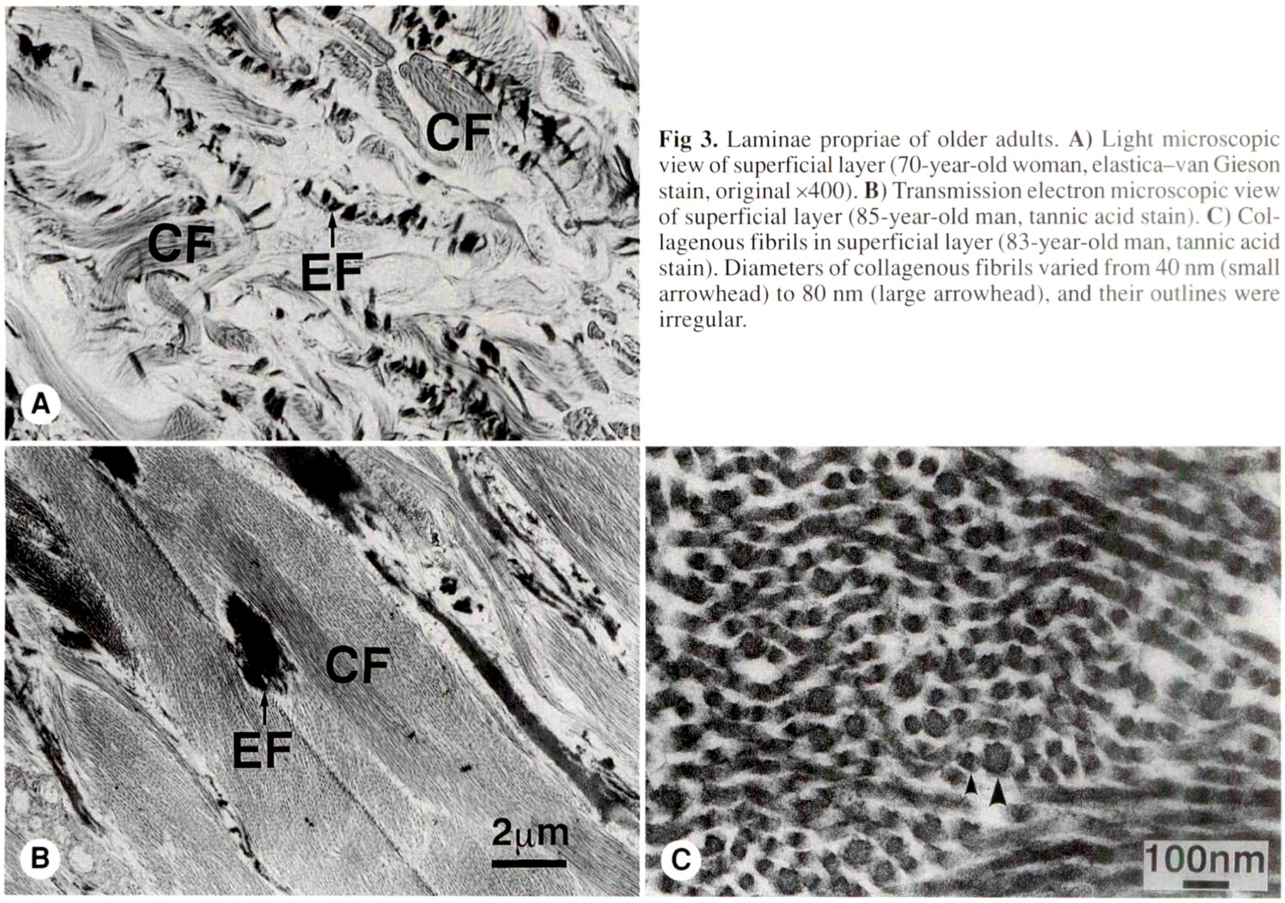

Fig 3. Laminae propriae of older adults. **A)** Light microscopic view of superficial layer (70-year-old woman, elastica–van Gieson stain, original ×400). **B)** Transmission electron microscopic view of superficial layer (85-year-old man, tannic acid stain). **C)** Collagenous fibrils in superficial layer (83-year-old man, tannic acid stain). Diameters of collagenous fibrils varied from 40 nm (small arrowhead) to 80 nm (large arrowhead), and their outlines were irregular.

dense in the deep layer of the lamina propria.

Superficial Layer of Lamina Propria of Aged Adult Vocal Fold Mucosa. Figure 1B shows a schema of the collagenous and reticular fibers and other extracellular matrices in the superficial layer of the lamina propria of an aged adult vocal fold mucosa.

In the aged vocal fold mucosae, collagenous fibers increased and reticular fibers decreased, especially in men, in the superficial layer of the lamina propria (Fig 1B). The collagenous fibers formed bundles and their density was high (Fig 3A,B). The collagenous fibril diameters differed (40 to 80 nm), and their outline was irregular (Fig 3C). Twisted collagenous fibrils were present (Fig 3C). As the collagenous fibers increased, the spaces among the collagenous fibers and the interstitial spaces for the other extracellular matrices decreased (Figs 4 and 5).

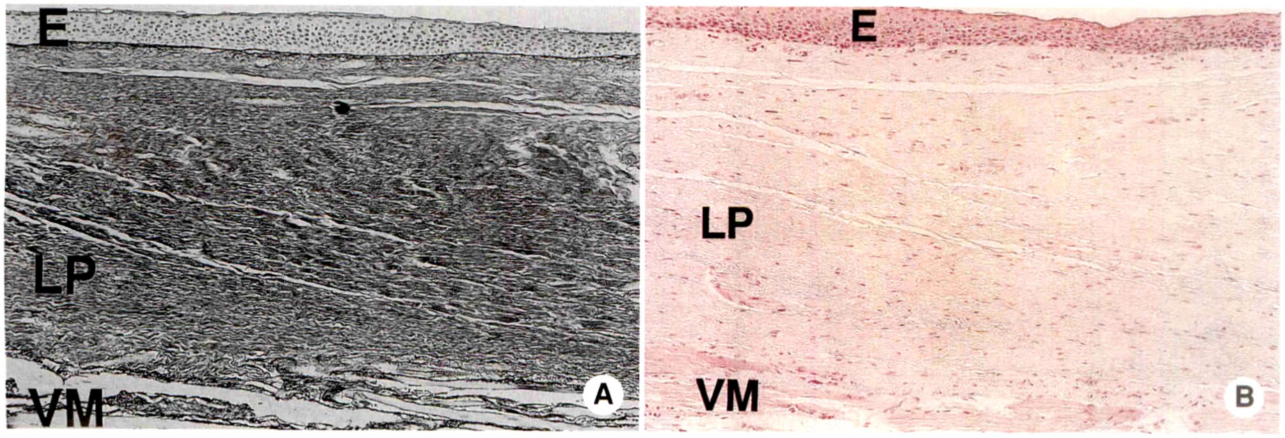

Fig 4. Horizontal sections of vocal fold (85-year-old man, original ×50). E — epithelium, LP — lamina propria of vocal fold mucosa, VM — vocalis muscle. **A)** Collagenous fibers are increased from deep layer to superficial layer of vocal fold mucosa (silver stain). There are few spaces among collagenous fibers. **B)** Spaces among collagenous fibers and interstitial spaces are decreased (Alcian blue stain). Some glycosaminoglycan, stained light blue, can be detected.

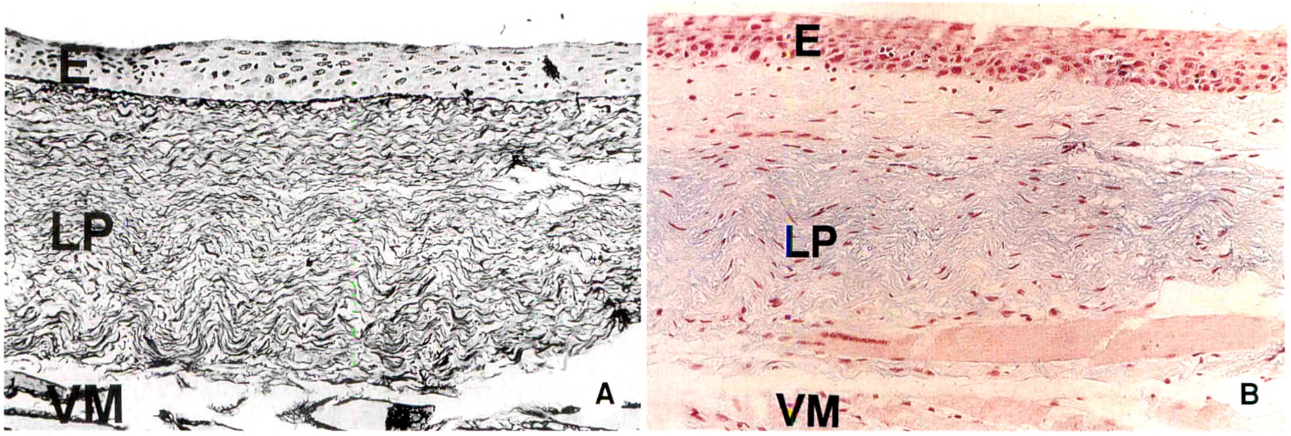

Fig 5. Horizontal sections of vocal fold (82-year-old woman, original ×100). **A)** Collagenous fibers are increased in lamina propria, but there are spaces among collagenous fibers (silver stain). **B)** There are spaces among collagenous fibers and interstitial spaces (Alcian blue stain). Glycosaminoglycan, stained light blue, can be detected.

Intermediate and Deep Layers of Lamina Propria of Aged Adult Vocal Fold Mucosa. Collagenous fibers increased in the deep layer of the aged lamina propria (Fig 6). Occasionally, masses of dense collagenous fibers and fibrous tissue could be seen in the deep layer of the vocal fold mucosa (Fig 7). Occasionally, the collagenous fibers were increased from the deep layer to the superficial layer of the vocal fold mucosa, and consequently there were no layered structures (Fig 4A).

In the aged lamina propria of the vocal fold mucosa, not only changes in the 3-D structures of the collagenous and reticular fibers, but also their qualitative and quantitative changes, have an effect on the 3-D structure of the extracellular matrices.

DISCUSSION

The supportive functions and viscoelasticity of the connective tissue depend largely on the properties of its extracellular matrices. The main extracellular matrices of the vocal fold mucosa are reticular fibers, collagenous fibers, elastic fibers, glycoprotein, and glycosaminoglycan (proteoglycan).[2,3]

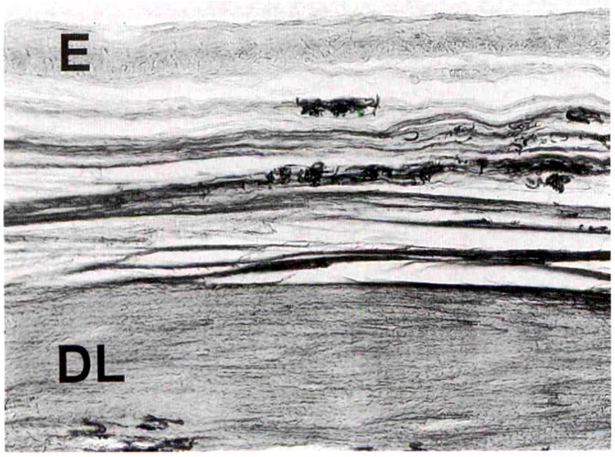

Fig 6. Horizontal section of vocal fold (78-year-old woman, elastica–van Gieson stain, original ×100). Collagenous fibers are increased in deep layer of lamina propria (DL). E — epithelium.

Reticular and collagenous fibers are essentially identical biochemically,[1] and have been found to have different morphological forms of the same fibrous protein, collagen.[1] Collagenous fibers are flexible, but offer great resistance to any pulling force.[1] The fibrils of fibrillar collagen, such as reticular and collagenous fibers, provide tensile strength and resilience and serve as stabilizing scaffolds in the extracellular matrices.[4]

The predominant type of collagen in the lamina propria of the vocal folds is type III.[5] Type III collagen is one of the fibrillar collagens,[4,6,7] and appears to be a major constituent of the slender 50-nm fibers that have traditionally been designated as reticular fibers.[1] Reticular fibers have been found in the superficial and intermediate layers of the lamina propria of the vocal fold mucosa.[2] Also, reticular fibers are most abundantly seen around the vocal fold edge.[2] These distributions of the reticular fibers indicate that the reticular fibers are located at the portion of the vocal fold mucosa that vibrates the most.[2] Reticular fibers are thin and result in a more compliant tissue.[7] The function of reticular fibers is structural maintenance in expansible organs.[1] The reticular fibers in the vocal fold mucosa are composed of slender fibrils, and those fibrils do not form bundles, but rather, form delicate 3-D networks. The spaces among the fibers are relatively large and possess innumerable potential spaces.[2] These extracellular interstitial spaces are made up of minute chambers or compartments occupied by other extracellular matrices.[2] Glycoprotein and glycosaminoglycan (proteoglycan) are situated among the spaces of the reticular fibers, and elastic fibers run through the spaces.[2] The delicate

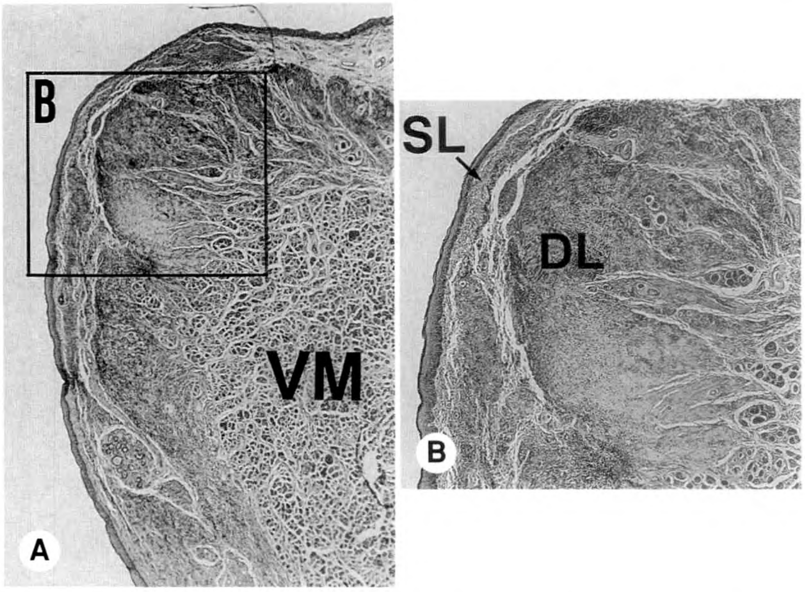

Fig 7. Coronal section of vocal fold (97-year-old man, elastica–van Gieson stain). **A)** Original ×12.5. VM — vocalis muscle. **B)** Magnification of region shown in A (original ×25). Collagenous fibers are increased and their density is high in superficial layer (SL); this layer is thin. Masses of dense collagenous fibers and fibrous tissue can be seen in deep layer (DL).

3-D structure of the reticular fibers contributes to maintaining the structure of the vocal fold mucosa during vibration without disturbing the vibration.[2]

Adult vocal folds have a layered structure consisting of the epithelium; the superficial, intermediate, and deep layers of the lamina propria; and the vocalis muscle.[8,9] These layers can be divided into 3 sections: a cover, consisting of the epithelium and the superficial layer of the lamina propria; a transition zone, consisting of the intermediate and deep layers of the lamina propria or vocal ligament; and a body, consisting of the vocalis muscle.[8,9] The superficial layer is referred to as Reinke's space, and has ground substance, sparse fibroblasts, and sparse elastic and collagenous fibers.[8,9] The intermediate layer is primarily made up of elastic fibers, whereas the deep layer is chiefly composed of collagenous fibers.[8,9] These layered structures are very important in vibration.[8]

Superficial Layer of Lamina Propria of Aged Adult Vocal Fold Mucosa. It is the superficial layer that vibrates most markedly during phonation. The viscoelastic properties of the lamina propria, especially the superficial layer, are very important for the vibratory behavior of the structure.

Sato et al[10] carried out a light microscopic investigation of collagenous fibers in the superficial layer of aged vocal folds and observed that the density of collagenous fibers increased with age, especially in men. Hammond et al[11] made computerized quantitative measurements of the collagen distribution in the lamina propria with respect to age and gender. In this study, collagenous fibers increased and reticular fibers decreased in the superficial layer of aged vocal fold mucosa. The collagenous fibers formed bundles and their density was high, especially in men. These 3-D structures could not serve as flexible stabilizing scaffolds in the extracellular matrices. On transmission electron microscopy, the collagenous fibril diameters differed and the outline appeared irregular. Twisted collagenous fibrils were present. These findings indicated that not only changes in the 3-D structure of collagenous fibers, but also qualitative and quantitative changes, have occurred.

The spaces among collagenous fibers and the interstitial spaces for other extracellular matrices, such as glycosaminoglycan (proteoglycan) and elastic fibers, decrease with age in the superficial layer of the vocal fold mucosa. One of the roles of glycosaminoglycan (proteoglycan) is to give viscosity to tissues.[6] It is supposed that the aged vocal fold mucosae have less viscosity because of the decrease of glycosaminoglycan (proteoglycan). The main role of the elastic fibers is to give elasticity and resilience to the tissues.[7] The elastic fibers in the superficial layer of the lamina propria of vocal folds change morphologically with age, and the metabolism of these fibers is disturbed.[12] These elastic fibers appear to lose their function, no longer giving sufficient elasticity and resilience to the tissue.[12] With aging, the complex of the collagenous fibers and other extracellular matrices in the superficial layer of the vocal fold mucosa seems to change the viscoelastic properties of the lamina propria. It is supposed that geriatric changes in the fine structure of the superficial layer of the lamina propria are one of the important causes of the voice's changes with aging.

Intermediate and Deep Layers (Vocal Ligament) of Lamina Propria of Aged Adult Vocal Fold Mucosa. Hirano et al[13] noted that in men, the deep layer tended to become thicker with age, being associated with an increase in collagenous fiber density. In this study, the collagenous fibers increased and formed bundles, and their density was high in the deep layer of the lamina propria of the aged vocal fold mucosa. The collagenous fibril diameters differed, and the outline was irregular. Twisted collagenous fibrils were present. Occasionally, masses of dense collagenous fibers and fibrous tissue could be seen in the aged vocal fold mucosa. These masses seem to disturb the vibration of the vocal fold mucosa.

Destruction of Layered Structure in Aged Adult Vocal Fold Mucosa. As mentioned above, the layered structure of the vocal fold is very important in vibration.[8] In aged vocal fold mucosa, the collagenous fibers were occasionally increased all the way from the deep layer to the superficial layer, and consequently there was no layered structure. These vocal fold mucosae seem not to have the proper viscoelasticity.

The maculae flavae have been shown to control the synthesis of fibrous components such as the vocal ligament of the human vocal folds.[14-16] There is no structure that can be called the vocal ligament in newborns.[9,17,18] Only a few fibers run in the mucosa, and the entire lamina propria of the mucosa is loose in structure.[9,17,18] The maculae flavae are essential for the growth and development of the vocal fold mucosa.[17,19-21] Age-related functional changes in the maculae flavae are probably related to the age-related changes in the collagenous fibers in the vocal fold mucosa.

Not only changes in the 3-D structure of the collagenous and reticular fibers, but also their qualitative and quantitative changes, have an effect on the 3-D structure of the extracellular matrices. In addition, age-related changes in the other extracellular matrices among the collagenous and reticular fibers influence the 3-D structure of the extracellular matrices. Thus, viscoelasticity must change to ensure normal phonation, and this change in viscoelasticity would explain one component of aging of the voice.

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REVERSIBLE LATERALIZATION OF THE PARALYZED VOCAL CORD WITHOUT TRACHEOSTOMY

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BUDAPEST, HUNGARY

The initial management of bilateral abductor vocal cord paralysis is usually tracheostomy. It is proposed that a reversible endoscopic vocal cord lateral fixation would avoid this morbid procedure. The operation is performed by laryngoscopy utilizing the endo-extralaryngeal suture technique of Lichtenberger. Two polypropylene sutures are looped over one of the paralyzed vocal cords and brought out through the neck skin. A small incision is made, and the sutures are secured in the sternohyoid muscle. If movement of one or both vocal cords returns, the sutures are removed. Sixty-one of 63 cases were successful. In 53 cases, the airway became stable, without return of function. In 8 cases, one or both of the vocal cords became mobile 3 to 4 months after the operation. The reversible endo-extralaryngeal lateralization of the vocal cord using the above suture technique ensures a stable airway immediately. This technique avoids the need for tracheostomy in cases of bilateral abductor vocal cord paralysis.

KEY WORDS — endo-extralaryngeal suture technique, lateralization, paralysis, tracheostomy, vocal cord.

INTRODUCTION

Reports in the literature indicate that vocal cord paralysis will frequently occur after thyroid surgery and will less often occur in other disorders such as viral infection. In particular, bilateral recurrent laryngeal nerve palsy occurs after major surgery for malignant thyroid disease. If the surgeon is not able to preserve the recurrent laryngeal nerves in performing total thyroidectomy, paralysis of the vocal cords is almost always present.

Most patients will require immediate intervention after the onset of recurrent laryngeal nerve injury, if the vocal cords are in the paramedian position. Some patients are able to tolerate a mild to moderate dyspnea for a long period of time without intervention to protect the airway. Patients who need immediate intervention frequently undergo tracheostomy as the first treatment option. Tracheostomy in many instances is a morbid and unpleasant sequel of vocal cord paralysis, and the patients will frequently have the tracheostomy for at least 6 to 12 months or longer, with many traumatic psychological side effects. The long-term complications of tracheostomy are obvious and well known to people who manage these patients. Tracheostomy increases the cost and length of hospitalization and also makes the outpatient ambulatory care more expensive and difficult. Many procedures have previously been described for improving the airway in bilateral vocal cord paralysis. These include endolaryngeal arytenoidectomy,[1,2] endolaryngeal arytenoidectomy with submucosal cordectomy,[3,4] and transverse cordotomy.[5,6] These procedures cause tissue damage and irreversible changes in the larynx. Reinnervation procedures have been attempted, but the success rate of these, even in the best of hands, has not been overwhelming.[7] There are published reports of an extra-endolaryngeal suture technique for bilateral vocal cord paralysis.[8-11]

Early work carried out in laboratory experiments on animals (in the years 1977 to 1980) involved improvement of the laryngeal airway that would be suitable for reversible and irreversible paralysis. The first and most important step was to develop a new *endo-extra*laryngeal suture technique and a new device, the endo-extralaryngeal needle carrier instrument.[12-16] This instrument has been used to improve the airway in bilateral vocal cord paralysis by an endo-extralaryngeal suture technique,[4,12,14-16] to place anterior commissure keels in the case of anterior commissure stenosis,[13,16,17] and to place stents endoscopically without the use of blind methods.[16,18] The purpose of this presentation is to add another indication for use of the endo-extralaryngeal needle carrier to maintain the airway immediately after bilateral vocal cord paralysis without the use of a tracheostomy.

PATIENTS AND METHODS

Sixty-three patients were operated on for bilateral vocal cord paralysis between January 1980 and December 2000 by the reversible endo-extralaryngeal lateralization procedure. The causes of the paralysis, methods of airway management used, and fol-

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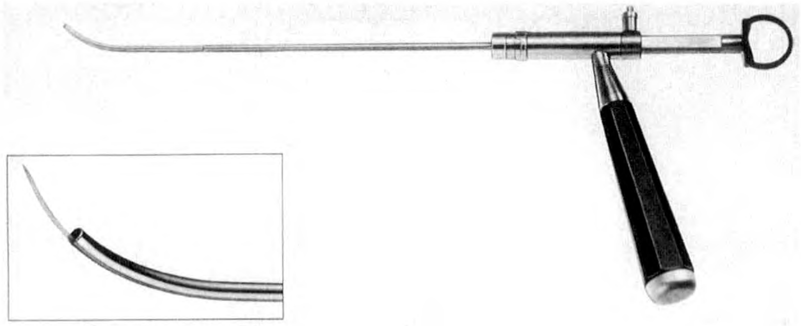

Fig 1. Endo-extralaryngeal needle carrier instrument developed by G. Lichtenberger.

low-up data were recorded.

SURGICAL TECHNIQUE

In the beginning, the procedure was performed under general anesthesia via orotracheal intubation. Initially, it was thought that the intubation tube complicated the procedure of placing the lateralized sutures in the larynx. In the first years after 1980, some patients underwent the operation with this technique[12,15] under special anesthesia using a combination of gamma hydroxy-butyrate and diazepam.[19] With this type of anesthesia and no tracheostomy, the operations could be performed without any problems; however, because of the anesthetic's long-lasting effects, patients required 4 to 5 hours of intensive postoperative care. Most of the patients were operated on in the past 5 years, when high-frequency jet anesthesia was available. Some of the patients presented were already orally intubated. The tube had been removed in the operating room, and jet anesthesia was introduced for glottis dilation without tracheostomy.

Lateral fixation sutures were placed through the larynx with the help of the endo-extralaryngeal needle carrier (Fig 1). The skin was cut in the same line of the previous incision for thyroidectomy. Only the skin and platysma flap were identified and retracted. The microlaryngoscope was then introduced. The paralyzed vocal cords were brought into the field of vision (Figs 2A and 3A). One end of the lateral fixation suture was pushed through the larynx below the posterior third of the vocal cord (Fig 2B). A similar stitch was made, this time above the posterior third of the vocal cord (Fig 2C), and the ends of the fixation suture were pulled and knotted over a small silicone sheet placed on the thyroid cartilage or on the sternohyoid muscles (Fig 2D). Formerly, we used the silicone sheet only if the sternohyoid muscle had been damaged during thyroid surgery. The second lateral fixation suture was placed in similar fashion 1 to 2 mm anterior to the first suture (Figs 2E and 3B). The knotted suture over the sternohyoid muscle was then left subcutaneously, and the previously opened wound was closed. In the other version, the assistant surgeon made an approximately 10-mm-long incision between the two ends of the thread, which were pushed through the larynx and neck skin; then, both ends were pulled back under the skin and tied above the prelaryngeal muscles.[20] Finally, the skin incision was closed with sutures. The patients were placed in

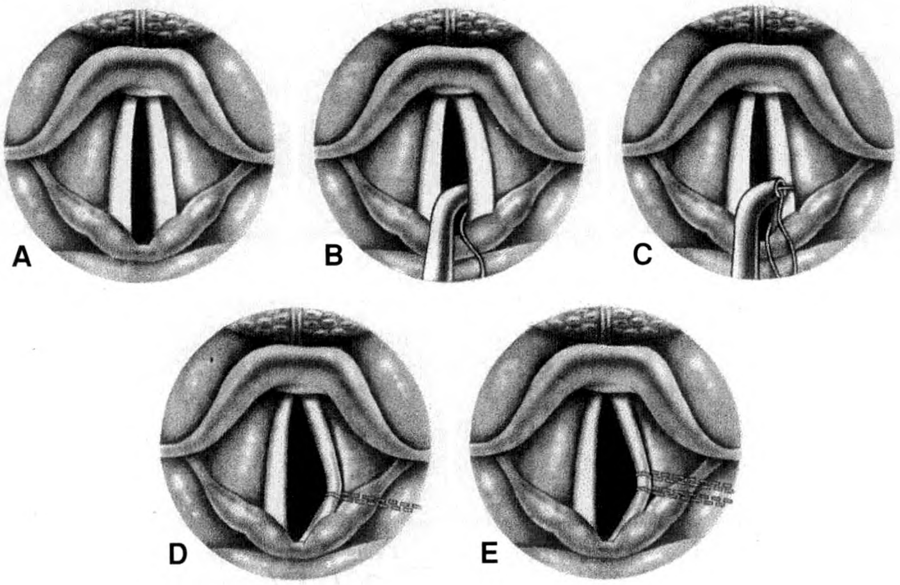

Fig 2. Stages of reversible lateralization of paralyzed vocal cord. See text for explanation of **A-E**.

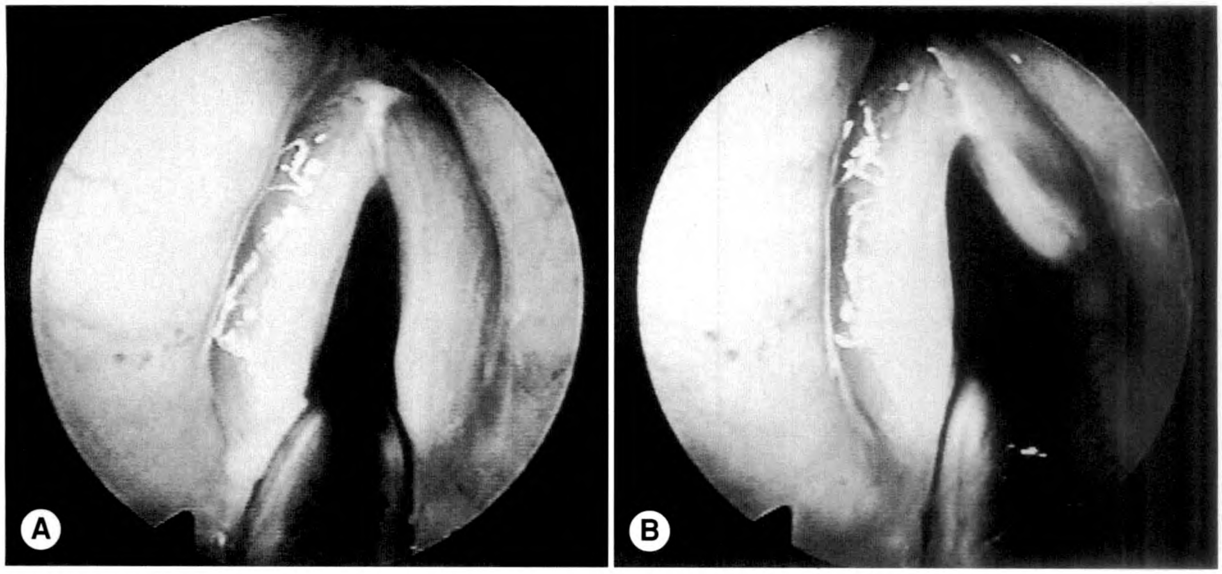

Fig 3. **A)** Paralyzed vocal cords under high-frequency jet anesthesia. Small jet catheter is between vocal cords. **B)** Right vocal cord has had temporary lateral fixation.

the intensive care unit immediately after operation after spontaneous breathing returned. The patients were discharged from the hospital within a few days, when it was thought that the airway was stable (Fig 4).

RESULTS

There were 57 female and 6 male patients who underwent this procedure. Their ages ranged from 7 to 79 years. The vast majority (57) of the patients had bilateral vocal cord paralysis following thyroid surgery. The other causes of the bilateral paralysis were surgery of the carotid artery in 2 patients, surgery of the esophagus for the management of a malignant tumor in 2 patients, congenital paralysis in 1 patient, and an unknown cause in 1 patient. The follow-up of the patients ranged from 4 months to 20 years. (Most of the patients were operated on in the past 5 years.) The operation was successful in preserving the airway without the need for a tracheostomy in 61 of the 63 patients. The function of the vocal cords did not return in 55 patients. Two of these patients had to undergo tracheostomy because the glottic chink was not wide enough even though the patient had a laterofixation. Two patients' vocal cords slightly medialized in a 4- to 6-month period after lateralization, and they had to undergo an irreversible lateralization procedure without tracheostomy. Eight patients had a return of vocal cord movement 3 to 4 months after the lateralization procedure. Six of these had a return of function unilaterally. Two had return of function of both vocal cords. In these patients, the lateralization suture was removed (Table 1). The postoperative vocal quality varied among patients. Lat-

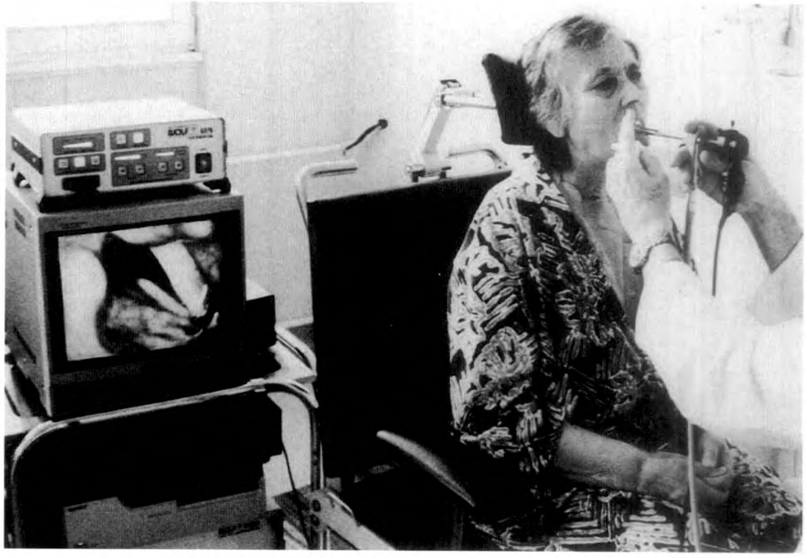

Fig 4. Patient 5 days after reversible lateralization of right vocal cord.

TABLE 1. RESULTS OF REVERSIBLE ENDO-EXTRALARYNGEAL LATERALIZATION IN 63 PATIENTS

	No. of Patients
Glottic chink was wide enough, but function of vocal cords did not return	51
Function of vocal cords returned on one side in 3 to 4 months	6
Function of both vocal cords returned in 3 to 5 months	2
Lateralized vocal cords were slightly medialized in 4 to 6 months; irreversible lateralization had to be performed as second procedure without tracheostomy	2
Tracheostomy had to be performed, because glottic chink was not sufficiently wide after reversible lateralization	2

Patient ages ranged from 7 to 79 years (57 female, 6 male). Follow-up ranged from 4 months to 20 years, with majority of follow-ups less than 5 years.

eralization influences voice quality somewhat. Those patients who had recovery in 3 to 4 months essentially recovered their normal voices after removal of the lateral fixation suture. Patients who did not recover muscular function had a slightly more dysphonic voice, although the voice quality in many of these patients remained quite good. There were temporary mild postoperative complications that all resolved spontaneously. Some patients had some pain on swallowing that was controlled by medication alone. Also, temporary mild aspiration occurred in some of the patients in the first postoperative days, especially in elderly individuals; it disappeared in all cases.

Eleven of the patients were evaluated before and after operation by spirometry. These were patients who were not orally intubated. The operation could be performed after the spirometric evaluation. The preoperative and postoperative results of spirometric evaluation were recorded (Table 2). The value of the forced inspiratory volume in the first second was specifically emphasized.

DISCUSSION

The endo-extralaryngeal suture technique and the needle carrier instrument previously described allow for excellent lateral fixation of paralyzed vocal cords. This is much easier to perform and more advantageous than the extra-endolaryngeal suture technique.[8-11] The main advantage of the endo-extralaryngeal technique is that the appropriate spot in which the needle is to be placed with the lateral fixation suture can be pushed through the larynx to an external position and be exactly noted with the use of the operating microscope.[16,20] This is compared with the somewhat blind procedure of the extra-endolaryngeal suture technique, in which the level of the vocal cords can only be estimated from the outside. Problems with anesthetic techniques for this procedure have progressed from the early days of this technique, beginning in 1980. To avoid use of a tube, special anesthesia[19] was used so that the needle carrier could be manipulated more easily in the larynx. It was thought that this was somewhat disadvantageous because of postoperative sedation and the need for observation in an intensive care unit. In recent years, jet ventilation has been used, and this has proven to be most advantageous in this procedure. Modern-day valved and bivalved adjustable laryngoscopes prove to be very advantageous and simplify the maneuvering of the needle carrier in the lumen of the larynx.

TABLE 2. RESPIRATORY FUNCTIONAL RESULTS AFTER REVERSIBLE ENDO-EXTRALARYNGEAL LATEROFIXATION WITHOUT TRACHEOSTOMY

Patient No.	*Age (y)*	*Sex*	*Vocal Cord Recovery*	*FIV1*	
				Preop	*Postop*
1	71	F	–	0.71	1.21
2	54	F	–	1.31	1.95
3	67	F	–	0.85	1.15
4	67	F	–	0.27	0.49
5	46	F	–	0.61	1.83
6	59	F	–	0.34	0.63
7	67	F	–	0.85	1.13
8	76	F	–	0.00*	1.33
9	63	F	–	0.21	1.42
10	58	F	–	0.26	0.69
11	56	F	–	0.85	1.97

FIV1 — forced inspiratory volume in first second.

*This value was obtained because machine stops when parameter is critically low.

In most cases, it is suggested that the initial suture be placed as far posterior on the vocal cord as possible, at a point very near the vocal process. It is not, however, always optimal to try to loop the suture around the vocal process, because of its very posterior position. The more posterior position of suture placement can be unsuccessful, because the thyroid cartilage is more dense in that area and the angulation is such that the suture will not pass through to the skin or subcutaneous areas. The other problem that can happen with the lateral fixation suture is that the suture may cut into and through the vocal cord. Two patients were operated on unsuccessfully after thyroid surgery and intubation for several days. Their vocal cords were damaged by the tube and became too soft to hold the lateral fixation sutures, which cut through. Accordingly, we emphasize that a vocal cord damaged by the tube is a contraindication to this reversible lateralization procedure.

In some cases, because of previous thyroid surgery, the sternohyoid muscle has been partially or totally removed. It is recommended to place a small silicone sheet at the level of the thyroid cartilage to protect it and prevent cutting of tissue with the sutures. Further considerations of this surgery are that the patient should be deeply anesthetized and paralyzed, because waking of the patient to the point that muscular function returns will cause edema of the larynx that might be of concern. Most of the time, the postoperative edema is only moderate and does not require further airway support or tracheostomy. Edema of the larynx can be greatly relieved by the use of steroids and anti-inflammatory derivatives. A broad-spectrum antibiotic can prevent infection of the stitching canal in the postoperative period.

This operation is best performed within 8 weeks after the onset of vocal cord paralysis. It is thought that lateral fixation of the vocal cord should be performed immediately after the identification of the patient's problem, to give the patient relief from the morbidity and the anxiety of waiting in hope of the return of vocal cord function, which, in most cases, as noted in this report, will not occur. Electromyography of the larynx will not give much information early in the postinjury period. In patients whose respiration is only mildly impaired and who do not have lateralization, one may be able to use electromyography as a prognosticator for recovery. If the electromyography does show denervation, then an irreversible definitive lateral fixation procedure should be performed. It is thought that early lateralization of the vocal cords is particularly helpful in elderly patients, who suffer from much more stridor and dyspnea if both vocal cords are paralyzed. These patients do not have strong intercostal and diaphragmatic drive and therefore tend to have more respiratory difficulty. The operation, even in an aged patient, is very short. The only problem is that an older patient might have temporary aspiration after the procedure.

Endo-extralaryngeal suture placement has been used in patients with chronic vocal cord paralysis with very good results. In acute vocal cord paralysis, this procedure appears more effective than previous techniques. In addition to avoiding tracheostomy, it enabled return of vocal cord function in a certain percentage of our patients. In 2 cases, the vocal cords recovered on both sides. The vocal cords can be lateralized as long as they are nonfunctional. After recovery, the power of these small muscles is very strong and overcomes the effect of the lateral fixing thread. The vocal cords became mobile before the removal of the lateral fixation sutures.

CONCLUSIONS

1. Temporary, reversible lateral fixation of paralyzed vocal cords is a reliable method for management of acute bilateral vocal cord paralysis.

2. This procedure is minimally invasive, and in patients in whom function returns, it is reversible without damaging the structure of the vocal cord.

3. This method is advantageous because tracheostomy is avoided in most cases, together with the associated morbidity and complications.

4. A single procedure ensures an immediate sufficient airway without causing morbidity and distress for the patient.

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SUBMANDIBULAR DIAGNOSTIC AND INTERVENTIONAL SIALENDOSCOPY: NEW PROCEDURE FOR DUCTAL DISORDERS

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We present our initial experience with submandibular sialendoscopy, a new therapeutic approach for disorders of Wharton's duct. We review the sialendoscopes used and discuss their respective merits. We evaluated and treated 129 consecutive patients with suspected ductal disorders. Diagnostic sialendoscopy was used for classifying ductal lesions as sialolithiasis, stenosis, sialodochitis, or polyps. Interventional sialendoscopy was used to treat these disorders. The type of endoscope used, the type of sialolith fragmentation and/or extraction device used, the total number of procedures, the type of anesthesia, and the number and size of the sialoliths removed were the dependent variables. The outcome variable was the endoscopic clearing of the ductal tree and resolution of symptoms. Diagnostic sialendoscopy was possible in 131 of 135 glands (97%), with an average (±SD) duration of 28 ± 15 minutes. Interventional sialendoscopy was attempted in 110 cases, with an average duration of 71 ± 41 minutes, with a success rate of 82%. Multiple sialendoscopies were necessary in 25% of cases. General anesthesia was used in 12% of cases. Submandibular gland resection was performed in 4%. The average size of the stones was 4.9 ± 2.9 mm. Multiple sialoliths were found in 31 cases (29%). Sialolith fragmentation was required in 26%. Larger and multiple stones often required longer and multiple procedures and general anesthesia, and more often resulted in failures. Semirigid endoscopes had a higher success rate (85%) than flexible sialendoscopes (54%). Complications were mostly minor, but were encountered in 10% of cases. Diagnostic sialendoscopy is a new technique for evaluating salivary duct disorders that is associated with low morbidity. Interventional sialendoscopy allows the extraction of sialoliths in most patients, thus preventing open gland excision.

KEY WORDS — salivary duct, sialendoscopy, sialolith, Wharton's duct.

INTRODUCTION

Salivary gland disorders are traditionally divided into neoplastic and non-neoplastic disorders, the latter being further subdivided into inflammatory and noninflammatory ones.[1,2] The advent of new endoscopic techniques[3,4] allows a complete exploration of the salivary ductal system and a precise evaluation of its disorders. Therefore, the present study supports the division of non-neoplastic salivary gland diseases into parenchymal ones, which require traditional treatments, and ductal ones, which can be handled endoscopically in the majority of cases.

Most salivary ductal disorders are obstructive, with sialolithiasis being the most frequent cause, although its exact incidence is unknown. Treatment of distal submandibular sialolithiasis has been performed for many years by simple anterior marsupialization (sialodochotomy and/or sialodochoplasty)[5] of Wharton's duct, which allows the direct removal of the stone. The classic approach to proximal sialolithiasis is antibiotic and anti-inflammatory treatment, in hope of a spontaneous stone extrusion through the papilla. Recurrent episodes of sialadenitis are seen as an indication for open submandibular gland extirpation, although the precise indications remain ill defined. Interestingly, sialolithiasis is the most frequent reason for submandibular gland resection.[6,7]

Sialendoscopy[4,8,9] is a new procedure for visualizing the lumen of the salivary ducts, as well as diagnosing and treating ductal diseases. Because of the required equipment and the complexity, the duration, and the potential complications of the procedure, it appears important to distinguish two different procedures: diagnostic sialendoscopy and interventional sialendoscopy.[10] Diagnostic sialendoscopy is an evaluation procedure, while interventional sialendoscopy must be considered as an operation on the obstructive ductal disease.

We report on the sialendoscopic evaluation and treatment of 129 successive patients with suspected ductal disease, performed by the first author in 2 university centers (Geneva University Hospital, Geneva, Switzerland, and Edouard Herriot Hospital, Claude Bernard University, Lyon, France).

From the Department of Otolaryngology–Head and Neck Surgery (Marchal, Dulguerov, Lehmann) and the Division of Diagnostic Radiology (Becker), Geneva University Hospital, Geneva, Switzerland, and the Department of Otolaryngology–Head and Neck Surgery, Edouard Herriot Hospital, University Hospital of Lyon, Lyon, France (Disant). Mr Barki is an advisor to Karl Storz GMBH, Tüttlingen, Germany.

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MATERIALS AND METHODS

Patients. Between November 1995 and March 2000, 129 consecutive patients with suspected submandibular duct dysfunction were evaluated. The average (±SD) age was 39 ± 16 years, with a range of 6 to 93 years. Six of the patients had bilateral symptoms, so 135 primary sialendoscopies were attempted.

Most of the patients had preoperative radiologic evaluation studies such as sialography, ultrasonography, magnetic resonance sialography,[11] and, rarely, computed tomography.

The observed disorders of Wharton's duct were classified as sialolithiasis, stenosis, sialodochitis, and ductal polyps. Each pathological finding in each ductal system was noted. In cases of multiple stones, the largest stone was measured with calipers.

The numbers of diagnostic and interventional procedures per gland were recorded, as well as the type of anesthesia (local versus general), the fragmentation and extraction device used (see below), and the number of open submandibular gland resections performed. "Sialendoscopic success" was recorded when the entire Wharton's duct and its primary branches were rendered free of disease. "Sialendoscopic failures" were recorded when sialendoscopy was impossible or unsuccessful, or when an open submandibular gland resection was performed.

The pain experienced during the procedure by the last 80 patients of this series was evaluated with a 10-cm visual analog scale.

Statistical analysis for categorical and ordinal variables was performed with the χ^2 test, and continuous variables were analyzed with the t-test (2 groups) or the Kruskal-Wallis test (3 groups) as implemented by SPSS software (version 9.0, Chicago, Illinois).

Endoscopes. The technology of the endoscopes we used evolved in 4 steps: free optical fiber, flexible endoscope, and 2 generations of semirigid endoscopic devices of various diameters.

1. Initial trials were performed with free optical fibers, ranging from 0.5 to 0.8 mm, without a rinsing or a directional system. When visualized, the stones were extracted blindly with commercially available grasping baskets.[12]

2. The second group of endoscopes used were flexible, consisting of a 1.5-mm–outside diameter flexible endoscope and a 0.5-mm working channel. These flexible endoscopes had a directional system, and in 1996, we began to use a rinsing solution.[8]

3. The third system included 2 devices: a semirigid single-lumen device (external diameter of 1.3 mm) for diagnostic endoscopy, and a double-lumen device for interventional procedures. In the latter, one channel was 1.1 mm and the other was 0.8 mm, the total surface being 2.67 mm^2. In both instruments, a 1-mm semirigid endoscope (Karl Storz AG) was secured for visualization in one of the channels. The second channel of the double-lumen device was a working channel used to pass different instruments for sialolith fragmentation and/or retrieval.[12]

4. The final endoscopic system also includes 2 different devices (Karl Storz AG). The Marchal diagnostic sialendoscope resembles the single-lumen diagnostic device described above, with slight modifications (Fig 1A). The outside diameter is 1.3 mm and the cross-sectional area is 1.33 mm^2. The Marchal interventional sialendoscope is a double-lumen device, with one channel of 1.1 mm for the endoscope, and a working channel of 0.8 mm, which is used for custom-made baskets and/or laser fibers (Fig 1B). The overall cross-sectional area is 2.29 mm^2. The tip of the instrument has been beveled and blunted for easier cannulation of the duct. Both instruments were slightly bent to facilitate exploration of the ductal tree.

Other Materials. Other materials include a custom papilla dilator and customized grasping wire baskets (Karl Storz AG; Fig 1C). Fragmentation of larger stones was achieved in this study with the following devices: an electrohydraulic lithotriptor with a 500-µm probe (Calcutript, Karl Storz AG) and a 400-µm holmium laser probe (Coherent, Versapulse Select, Santa Clara, California).

Surgical Technique. Details of the surgical technique have been previously described.[3,10] The procedure is done in most cases under local anesthesia. Local anesthesia is achieved by contact anesthesia (10% Xylocaine-soaked pledgets) followed by infiltration of the tip of the papilla. In addition, intermittent rinsing through the endoscope is achieved with a local anesthetic solution (1:1 Xylocaine 2% and sodium chloride 0.9%) that provides slight dilation of the duct, cleansing of the endoscope tip, and removal of pus, debris, and occasionally blood.

Dilation of the papilla is performed with customized salivary dilators of increasing diameter, followed by the papilla dilator. Earlier, large sialodochoplasties of the papilla were performed, and the edges of the Wharton's duct were sutured to the surrounding floor of mouth mucosa. For the past 2 years, simple dilation of the Wharton's duct papilla is performed in the majority of cases, with rare patients requiring a mini-papillotomy (<5 mm).

The initial procedure is diagnostic sialendoscopy, allowing a minimally invasive but complete explo-

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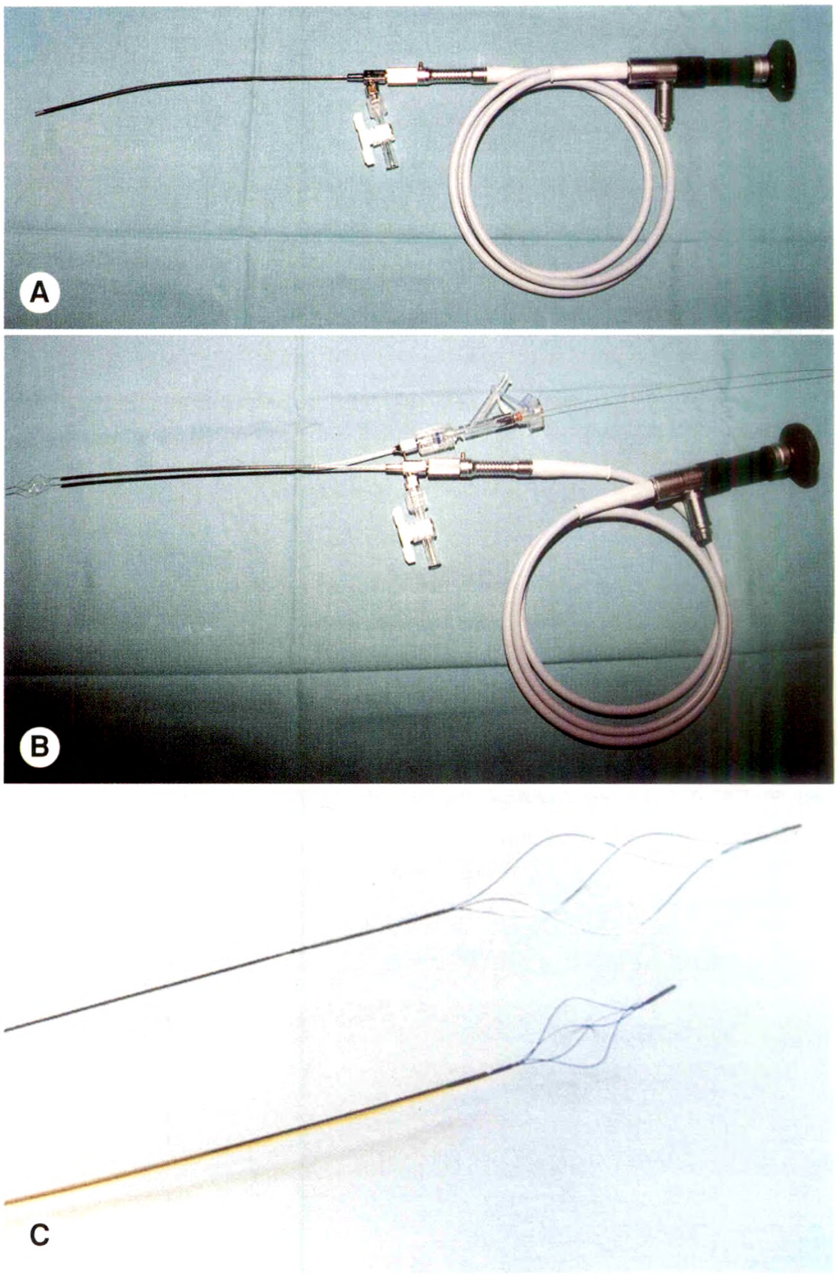

Fig 1. Equipment. **A**) Diagnostic sialendoscope. **B**) Interventional sialendoscope, with retrieving basket in working channel. **C**) Two retrieving baskets.

ration of the ductal system. When a stone or another ductal disorder is located, an interventional sialendoscopy is planned. For sialoliths smaller than 5 mm, it is performed during the same stage, with the interventional sialendoscope. The custom wire basket is passed behind the stone and deployed, the stone is trapped (Fig 2), and the whole sialendoscope is removed. For larger stones, fragmentation is required before extraction. As the use of the Calcutript and the use of the holmium laser under local anesthesia have proven to be painful, some sialolithotripsies were performed under general anesthesia. After the last stone is removed, the endoscope is introduced one last time to rinse the duct and verify its integrity.

The intervention is done under antibiotic prophylaxis. Oral antibiotics (amoxicillin–clavulanic acid or clindamycin) and corticosteroids (prednisone 50 mg/d) are administered for 48 hours. Frequent self-massages of the gland are recommended. A follow-up examination is performed 10 days after the procedure.

RESULTS

Diagnostic sialendoscopy was attempted in 135 submandibular glands suspected to have an obstructive process of the Wharton's duct. Diagnostic sialendoscopy was possible in 131 ducts (97%); the 4 failures were in 2 patients with a complete fibrosis of Wharton's duct and in 2 patients presenting a spontaneous perforation of the duct in the posterior floor of the mouth after an acute episode of sialadenitis.

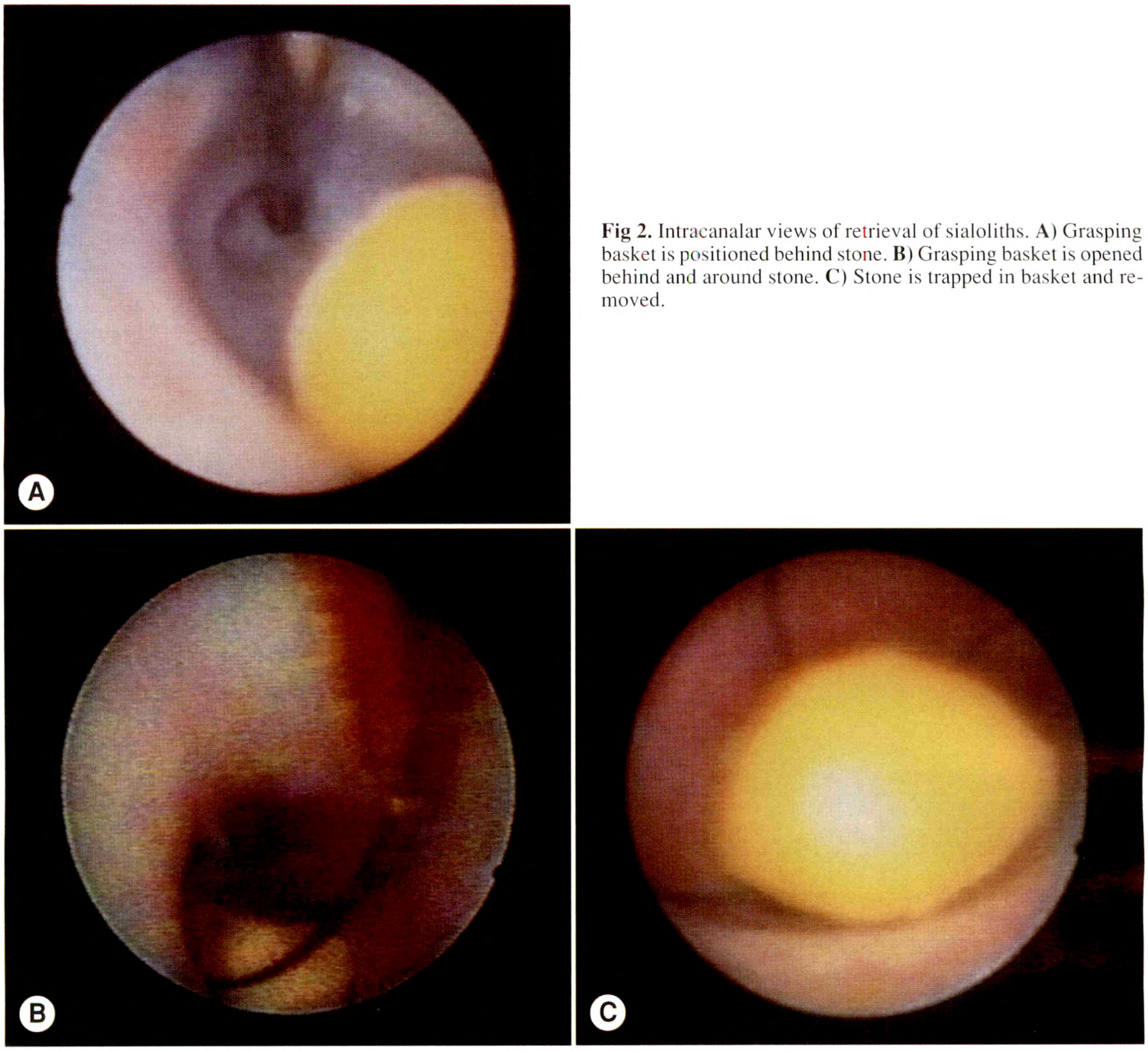

Fig 2. Intracanalar views of retrieval of sialoliths. **A)** Grasping basket is positioned behind stone. **B)** Grasping basket is opened behind and around stone. **C)** Stone is trapped in basket and removed.

The sialendoscopic findings of the 131 Wharton's ducts explored include 106 cases of sialolithiasis (74.1%), 15 cases of sialodochitis (10.5%), 8 cases of stenosis (5.6%), and 14 normal ducts (9.8%). No Wharton's duct polyps were identified. In 12 cases (8.4%), a combination of 2 of the above disorders was found. The average duration of diagnostic sialendoscopy was 28 ± 15 minutes. No complications were encountered during diagnostic sialendoscopy. The mean value of the pain experienced during the procedure was 2.4 ± 1.9 (minimum 0, maximum 10).

Interventional sialendoscopy, as well as diagnostic sialendoscopy, was performed over a 5-year period. The follow-up ranged from 3 months to 5 years; the mean follow-up was 2.75 years.

Submandibular sialendoscopy was attempted in 110 cases of sialolithiasis and stenosis. The average duration of the procedure was 71 ± 41 minutes. More than 1 interventional sialendoscopy was necessary in 28 cases (25%), resulting in sialendoscopy failures in 13 cases (47%). In 13 cases (12%), the procedure was performed under general anesthesia. Interventional sialendoscopy was successful in relieving the ductal obstruction in 90 cases, for an overall success rate of 82%. In the remaining 20 cases, failures were due to sialoliths embedded in the ductal wall in 14 cases, unsuccessful dilation of ductal stenosis in 4 cases, and impossibility of retrieving intraparenchymal stones in 2 cases. In 5 of these 20 cases, a submandibular gland resection was performed. The overall rate of submandibular gland resection was 4%.

Considering only sialolithiasis, multiple stones (Fig 3) within Wharton's duct were found in 31 cases (29%), with 2 sialoliths retrieved in 13 cases, 3 in 10

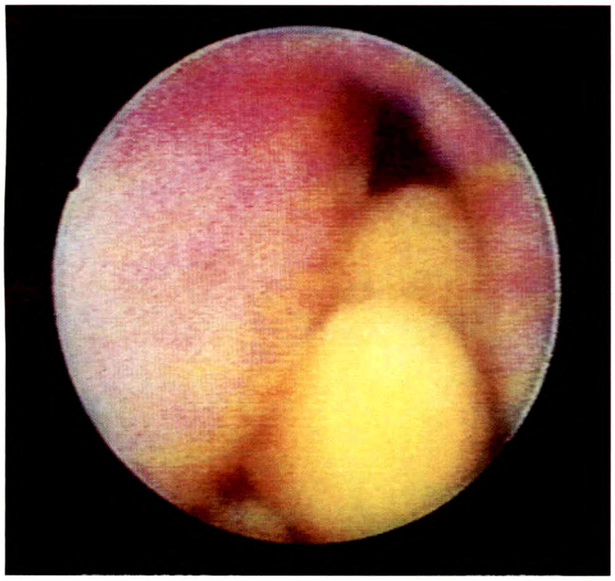

Fig 3. View of multiple intracanalar sialoliths.

cases, 4 in 5 cases, 5 in 2 cases, and 6 in 1 case. The average number of retrieved sialoliths per case was 1.56 ± 1.06. The presence of multiple sialoliths was statistically correlated to long operations, multiple procedures, and sialendoscopy failures (Table 1).

The average size of the submandibular sialoliths was 4.9 ± 2.9 mm. The presence of larger sialoliths was statistically related to procedures performed under general anesthesia, long operations, multiple procedures, use of fragmentation, and sialendoscopy failures (Table 2).

Stones larger than 4 mm were found in 43 cases (41%). Fragmentation prior to fragment extraction was required in 28 cases (26%), while the remaining 15 cases underwent a retrieval of the intact stone with the grasping wire basket. In the fragmentation group, an electrohydraulic device was used in 11 cases, and we were able to achieve complete clearance of the duct in 4 cases (36%). The use of a holmium laser for fragmentation in the other 17 cases was successful in 12 cases (71%; Fig 4).

In the remaining group of 15 patients, extraction of the stone with the grasping basket was successful in 9 cases (60%), although it resulted in canal perforations in all 9 cases, due to canal wall stripping (Fig 5). Two of these patients required hospitalization and intravenously administered corticosteroids and antibiotics because of remarkable submandibular swelling.

Complications of interventional sialendoscopy included ductal wall perforation in 11 cases and 2 wire basket blockages, for an overall complication rate of 10%. Nine of the 11 perforations are discussed above, and 2 perforations occurred with the holmium laser. One resolved with conservative therapy, but the second patient (12-mm stone, partially fragmented) experienced residual Wharton's duct obstruction followed by an acute blockage that required firm traction under sedation to free the instrument. Fragmentation under general anesthesia was required at a later time, followed by a submandibular gland resection.

TABLE 1. RESULTS OF INTERVENTIONAL SIALENDOSCOPY IN CASES OF SINGLE VERSUS MULTIPLE SIALOLITHS

	Single Sialolith	*Multiple Sialoliths*	*p*
Number	75 (71%)	31 (29%)	
Average maximal size	4.4 ± 2.9	5.9 ± 3.1	.4
General anesthesia	6 (8%)	7 (23%)	.05
Duration of procedure	62 ± 33	99 ± 46	.006*
Multiple procedures	10 (13%)	17 (54%)	<.001*
Submandibular gland resection	3 (4%)	2 (6.5%)	.6
Sialendoscopy failures	8 (11%)	10 (32%)	.01*

Data are numbers of cases, except for average maximal size (millimeters) and duration of procedures (minutes).

*Statistically significant difference.

Finally, a comparison between flexible endoscopy and semirigid endoscopy (Table 3) shows overall success rates of 54% and 85%, respectively ($p < .05$).

The mean value of the pain experienced during the sialendoscopy was 2.8 ± 2.1 (minimum 0, maximum 10).

DISCUSSION

Diagnostic Sialendoscopy. While the anatomic description of the submandibular ducts by Wharton dates to the 17th century, the first attempts to visualize them with an endoscope were reported in the early 1990s.[13,14] The instruments used were single optic

TABLE 2. RESULTS OF INTERVENTIONAL SIALENDOSCOPY AS FUNCTION OF SIZE OF SIALOLITHS

	<4 mm	*4-7 mm*	*7.1-10 mm*	*>10 mm*	*p*
Number	63 (59%)	29 (27%)	5 (5%)	9 (9%)	
General anesthesia	0 (0%)	2 (7%)	3 (60%)	8 (89%)	<.001*
Duration of procedure	50 ± 21	95 ± 36	127 ± 50	124 ± 44	<.001*
Multiple procedures	4 (6%)	12 (41%)	5 (100%)	6 (67%)	<.001*
Fragmentation	1 (2%)	14 (48%)	5 (100%)	8 (89%)	<.001*
Submandibular gland resection	0 (0%)	2 (40%)	0 (0%)	3 (60%)	<.001*
Sialendoscopy failures	2 (3%)	5 (17%)	3 (60%)	8 (89%)	<.001*

Data are numbers of cases, except for duration of procedure (minutes).

*Statistically significant difference.

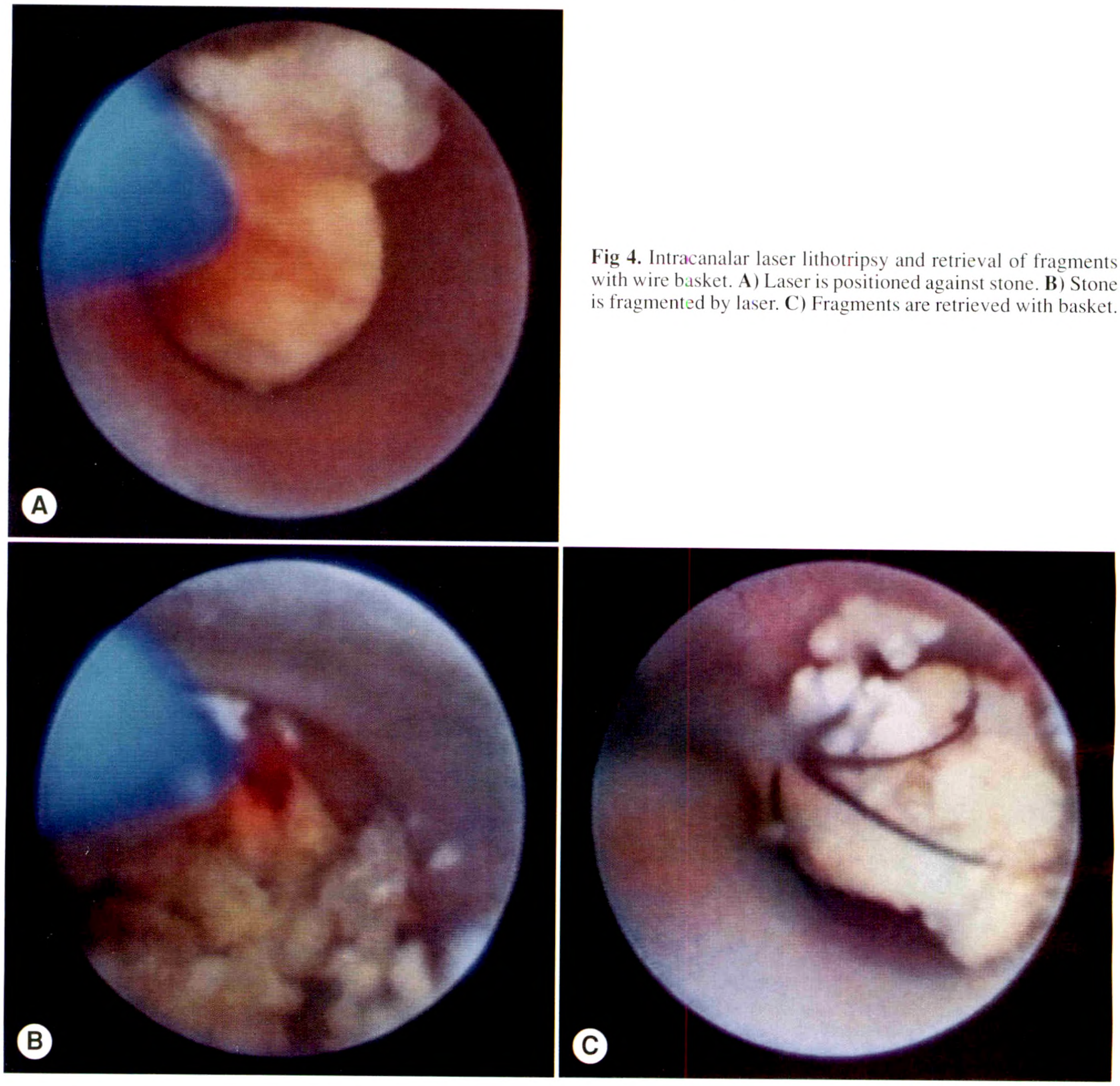

Fig 4. Intracanalar laser lithotripsy and retrieval of fragments with wire basket. **A)** Laser is positioned against stone. **B)** Stone is fragmented by laser. **C)** Fragments are retrieved with basket.

fibers similar to the ones we used at the beginning of this study. In our hands, visualization could be achieved only in the main duct, because of the absence of an orientation system and of a technique to dilate the duct. The resulting lack of good visualization makes these simple optic fibers disappointing even for diagnostic sialendoscopy, notwithstanding that any sialendoscopic treatment is performed blindly.[8]

Major progress was made with the introduction of a rinsing system, which provided for dilation of the duct, defogging, and irrigation of debris. Because rinsing requires a working channel, the principal limitation for diagnostic sialendoscopy becomes the diameter of the instrument. The flexible endoscope we used had a diameter of 1.5 mm, with a working channel of 0.5 mm and an optic fiber of 0.5 mm. This small diameter resulted in poor image quality and a short depth of field. Another problem was that to achieve a small outside diameter, the tip of the endoscope could be oriented only in one direction, requiring frequent 180° twisting of the entire fiberscope in order to advance in the ductal system. To be effective, this torsion had to be applied close to the papilla. Because the overall length of the fiberscope was 40 cm and the resistance of a fiber to torsion is directly proportional to its length, several fiberscopes were damaged. Because of similar experiences, Gundlach et al[15] have adopted 2-m-long flexible fiberscopes for salivary duct exploration and treatment.

In light of the advantages of rigid esophagoscopy, which allows straightening and stretching of the

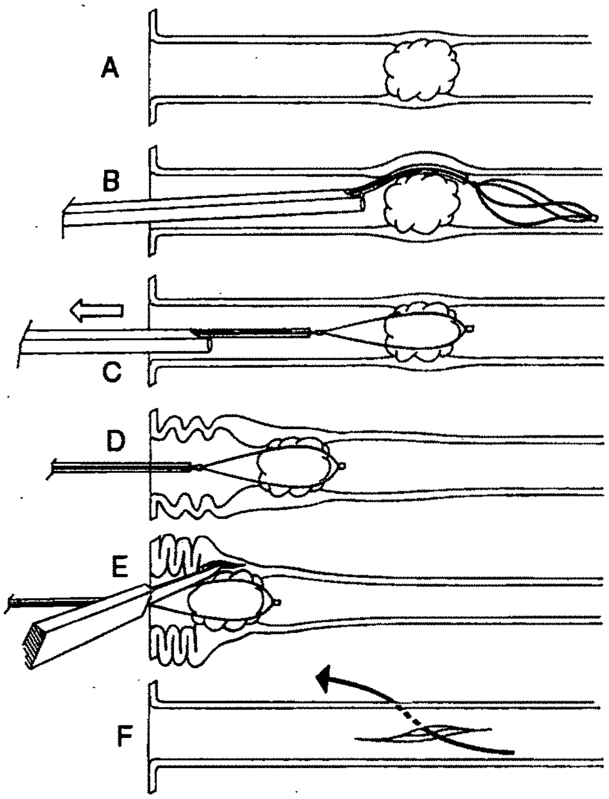

Fig 5. Drawing of mechanism of ductal perforation during interventional sialendoscopy. **A)** Large or embedded stone in salivary duct. **B)** Passage of grasping wire basket behind stone. **C)** Stone entrapment by instrument. **D)** Pulling on instrument to bring stone close to papilla. **E)** Sectioning of ductal wall required for sialolithiasis. **F)** Final situation, after duct retraction, with ductal wall perforation. In some cases, stone pulling, as shown in D, allows direct removal, but can result in stripping of ductal wall, with perforation also being final result.

esophagus, we designed a semirigid diagnostic sialendoscope with a 1.3-mm outside diameter. The main advantages of this instrument include a large optic fiber, which provides good illumination and a good image, and a rinsing system. However, as with any rigid endoscopy, special care should be taken to avoid trauma of the ductal walls and perforation. Progression should be done only in the center of the lumen, under clear vision.

Diagnostic sialendoscopy was possible in this study in 131 of the 135 cases (97%) under local anesthesia, with excellent patient tolerance (2.4 on pain scale of 0 to 10), even by children more than 10 years of age. (Younger children required general anesthesia.) The entire Wharton's duct could be explored and its disorders diagnosed with certainty. Since the endoscope is advanced in the center of the ductal lumen, under clear and direct vision, the associated morbidity remains minimal.

Sialendoscopy may be difficult and challenging for beginners, as any small movements of the sialendoscope against the canal wall result in a blurred image. Another difficulty of Wharton's duct endoscopy is the presence of a sphincter-like mechanism within the first 3 to 4 cm of the duct. This sphincter is hard to dilate, and its passage renders the beginning of the endoscopy difficult. Our experience differs from that of authors who have described a similar mechanism spanning the entire length of the duct.[16]

TABLE 3. RESULTS OF INTERVENTIONAL SIALENDOSCOPY ACCORDING TO TYPE OF ENDOSCOPE USED

	Free Optic Fiber	*Flexible Endoscope*	*Modified Fetoscope*	*Marchal Sialendoscope*	*p*
Number	11 (10%)	13 (12%)	27 (24%)	59 (54%)	
General anesthesia	0 (0%)	2 (15%)	2 (7%)	9 (15%)	.5
Duration of procedure	59 ± 29	92 ± 42	70 ± 36	70 ± 44	.2
Multiple procedures	1 (9%)	7 (5%)	5 (18%)	15 (25%)	.05
Submandibular gland resection	0 (0%)	2 (15%)	1 (4%)	2 (3%)	.2
Sialendoscopy failures	1 (9%)	6 (46%)	4 (15%)	9 (15%)	.045*

Data are numbers of cases, except for duration of procedure (minutes).

*Statistically significant difference.

Most of the patient population of this study had an extensive radiologic investigation before sialendoscopy, because it was ordered before their referral, or because of concomitant study protocols[11] (also Marchal et al, unpublished observations). At the present time, we do not require any specific radiologic examination before diagnostic sialendoscopy. We consider sialendoscopy to be the investigation of choice in the suspicion of salivary ductal diseases and any submandibular swelling of unclear origin.

The radiologic evaluation diagnosed sialodochitis in about 15% of patients, half of whom also had sialolithiasis. Endoscopy also revealed zones of the ductal wall that were erythematous, appeared inflamed, and often were associated with a canal narrowing. A frequent association with sialolithiasis was also noted. However, the correlation between radiology and endoscopy is not very good, as has been detailed elsewhere.[11] The clinical significance of sialodochitis requires further study. In particular, it is difficult to say whether this inflammation was part of the "initiation process" of calculus formation, or was secondary to an established sialolithiasis.

Interventional Sialendoscopy. The first report of a distal submandibular stone extraction was published in 1990; the procedure was performed blindly with a wire basket, during sialography.[17] Our initial endoscope was similar,[8] before technical developments allowed salivary stone extraction under endoscopic control.[8,9] The apparent high success rate with this technique (Table 3) results from case selection, by radiologic studies, of small and unique sialoliths located in the main duct. Although it is possible, we no longer use or recommend the semiblind technique with the grasping basket, in contradiction to authors who favor this equipment.[13,18] They present their technique as "endoscopic stone retrieval"; however, it should be clear from our description that during this technique, stone removal remains blind: an exploration of the duct with the optic fiber is followed by the removal of the endoscope and by the blind introduction of the retrieving basket. The potential dangers of this blind technique cannot be overemphasized.

We have also abandoned the use of flexible endoscopes for interventional sialendoscopy, not only because of difficult maneuvering and poor visualization, but also because of difficulty in sterilization of the material, and because the working channel of the fiberscope tends to become stripped by the grasping wire basket. The poor success rate of interventional sialendoscopy (Table 3) with this device might also be due to the particular fiberscope we used, as well as the lack of experience, since these cases were done early on.

Comparison of the 2 semirigid endoscopes does not show any clear advantage in terms of the final success of interventional submandibular sialendoscopy (Table 3). However, the main limitation is a larger diameter, which sometimes causes problems in Wharton's duct, and becomes a clear limitation for interventional sialendoscopy of the parotid gland.[19,20] Our newly developed sialendoscope was used in 59 patients with Wharton's duct sialolithiasis, with a success rate of 85%.

Sialoliths can be either directly extracted or fragmented before extraction. Prior publications have focused on fragmentation. The available techniques include use of electrohydraulic devices,[21-23] use of pneumoblastic devices,[24] external lithotripsy,[25] and intraductal laser lithotripsy.[14] A review of the advantages and disadvantages of these techniques can be found elsewhere.[10] In our opinion, the best technique is the fragmentation of sialoliths with a laser fiber, as initially described by Gundlach et al.[14] The laser fiber is introduced in the sialendoscope, laser sialolithotripsy is performed under direct visual control, and retrieval of stone fragments is achieved with grasping wire baskets. A distinct advantage of our technique is the retrieval of sialoliths and their fragments after lithotripsy, contrary to the majority of previously described methods.[14,21-25]

The holmium laser is well known and has proven efficiency for urolithiasis.[26,27] However, one has to be attentive to its potential dangers, because of its absorption characteristics in the surrounding tissues and because of the heat generated from the fragmentation within the narrow salivary ducts. The holmium laser should be used only under clear vision, tangential to the duct, and only in cases of sialolithiasis. The dye laser[14] has proven efficiency and low morbidity, because the high energy delivered is not absorbed by the tissues. Unfortunately, the cost of the device and its specificity may render its acquisition difficult. The combination of dye laser stone fragmentation and endoscopic retrieval is currently being investigated and might decrease the use of general anesthesia, and also further increase the success rate of interventional sialendoscopy.

We initially performed large sialodochoplasties as reported by others.[9,16] For the last 80 patients, we performed only a dilation, with minimal incision only for the stone retrieval. We found no residual stenosis on follow-up. Therefore, we advise against large marsupialization of the ductal papillae, in order to prevent retrograde passage of air and aliments.[10,28]

The results of interventional sialendoscopy were directly related to the size of the stone in the present study. Therefore, we propose the following approach. For stones smaller than 4 mm, interventional sialendoscopy should be performed immediately after the diagnostic procedure, in an ambulatory setting, under local anesthesia, without fragmentation, and with the grasping wire basket used for retrieval of the stone. For sialoliths larger than 4 mm, the shape of the stone is important, and an irregular shape or a very posteriorly located stone would render interventional endoscopy difficult, risking basket blockage or the necessity of using fragmentation procedures. In those cases, the procedure should be performed under general anesthesia.

Despite its apparent simplicity, interventional sialendoscopy is a technically challenging procedure. The maneuvering of the rigid sialendoscopes within the small salivary ducts requires extensive experience. Manipulation is delicate, and progression, which should remain absolutely atraumatic, might be hazardous because of the theoretical risks of perforation and vascular or neural damage. Significant trauma of the ductal wall could result in later stenosis. The necessity of performing the entire procedure under direct visualization cannot be overemphasized.

In 110 submandibular interventional endoscopies, we did not encounter any significant complications, such as damage to the facial nerve or lingual nerve, gross hemorrhage, or major canal wall perforations. Nevertheless, minor canal wall perforations were found in 11 cases, resulting in 1 submandibular gland resection and 2 admissions for intravenous treatment because of persistent swelling of the submandibular gland. In addition, blockage of the grasping basket is a rather traumatic experience for the patient and the surgeon, potentially resulting in emergency submandibular gland resection.

In conclusion, diagnostic sialendoscopy is a new, minimally invasive technique that may become the investigational procedure of choice for salivary duct disorders. Interventional sialendoscopy is an outpatient procedure performed under local anesthesia that allows the extraction of the majority of sialoliths and thereby prevents salivary gland excision. Further miniaturization of the instruments will allow fragmentation of larger stones and extraction of more deeply located stones.

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VIRTUAL LARYNGOTRACHEAL ENDOSCOPY BASED ON GEOMETRIC SURFACE MODELING USING SPIRAL COMPUTED TOMOGRAPHY DATA

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This prospective study describes the clinical utility of virtual endoscopy based on geometric surface modeling of the laryngotracheal lumen. Eighteen children with dyspnea related to either subglottic angioma ($n = 5$) or laryngotracheal stenosis ($n = 13$) were included. All patients underwent video-recorded operative endoscopy, spiral computed tomography, and 3-dimensional reconstruction of the laryngotracheal lumen. Modeling was achieved by contour detection on spiral computed tomographic images and reconstruction using a geometric shape-recognition algorithm. The generated surface was used for diagnosis and measurement using interactive and automatic tools. Findings of virtual endoscopy and operative endoscopy were compared. Virtual endoscopy confirmed narrowing of the airway in all cases. In 9 cases, high-grade stenosis prevented complete operative endoscopy, but virtual endoscopy allowed accurate assessment and measurement of the stenosis. The findings of operative and virtual endoscopy were concordant in 9 cases. We conclude that surface modeling provides valuable information for preoperative evaluation of laryngotracheal narrowing. The ability to assess extraluminal anatomy provides a clearer picture of overall disease involvement. In the future, virtual endoscopy will probably be used in conjunction with operative endoscopy for therapeutic decision-making. Noninvasive virtual endoscopy could become an alternative to traditional endoscopy under general anesthesia for therapeutic follow-up.

KEY WORDS — computer-assisted imaging, geometric modeling, laryngotracheal stenosis, subglottic angioma, virtual endoscopy.

INTRODUCTION

Pretherapeutic assessment of the glottis, subglottic region, and trachea is an essential phase of management of laryngotracheal disease in children. When practicable, operative endoscopy can confirm diagnosis by allowing direct visualization of lesions. However, in addition to being an invasive procedure requiring sedation, endoscopic assessment of involvement depends on the operator's experience.[1,2] Further evaluation is needed to determine the exact relationship of the lesion to cartilaginous structures, length of the stenotic region, and diameter of the residual lumen. Until now, these determinations have been made by computed tomography (CT). The recent advent of spiral CT has improved 2-dimensional (2-D) and 3-dimensional (3-D) reconstruction of the airway, a region prone to motion artifacts.

Three-dimensional reconstructions are typically based on either volume-rendering or surface-rendering techniques. Volume-rendering techniques include multiplanar reconstruction, maximum intensity projection, and the marching cube algorithm. These techniques are efficient for preserving the voxels of the interest area. Selection of the area of interest (segmentation) is obtained by analysis of density threshold levels.[3] These techniques have been used to produce virtual images for 3-D reconstruction[4] and endoscopy for preoperative assessment.[5,6] Surface-rendering techniques allow a significant reduction in the amount of data required by representing only the contours of structures in the form of facets[7] or continuous surfaces.[8] Using this approach, Fried et al[9] were able to generate virtual endoscopic images of the laryngotracheal lumen by meshing together triangles to depict anatomic features (lumen, arytenoid cartilage, vocal folds) and visualize the extraluminal anatomy by transparency. However, these images still required subjective interpretation, and objective measurement remained difficult. Neither volume rendering nor classical surface rendering takes into account geometric criteria such as shape, topology, or axis. In 1993, Sequeira et al[10] introduced a geometric approach based on mathematical modeling of tubular cavities. By this technique, the reconstructed model is built around an axis generated from 2-D objects and presents both continuous and curved properties. The purpose of this prospective clinical research study was to use this modeling technique to perform virtual endoscopy. Virtual and operative endoscopic

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PATIENTS AND RESULTS OF OPERATIVE AND VIRTUAL ENDOSCOPY

				Operative Endoscopy			*Virtual Endoscopy*	
Case	*Age (mo)*	*Sex*	*Disorder*	*Visualization*	*Narrowing (Grade*)*	*Length of Stenosis (mm)*	*Narrowing*	*Length of Stenosis (mm)*
1	7.1	F	Left posterolateral subglottic angioma	Complete	50% (I)	10	62%	12.6
2	5.6	F	Circular subglottic angioma	Incomplete	70% (I)	?	56%	14.2
3	37.7	F	Postintubation tracheal stenosis	Incomplete	90% (II)	?	86%	16.1
4	16.6	M	Postintubation subglottic stenosis	Complete	60% (I)	15	55%	14
5	3.2	M	Left subglottic angioma	Complete	85% (II)	10	76%	12.3
6	4.4	F	Right subglottic angioma	Complete	80% (II)	10	78%	12.1
7	4.1	M	Postintubation subglottic stenosis	Incomplete	95% (III)	?	100%	19.6
8	4.9	M	Congenital glottosubglottic stenosis	Complete	50% (I)	7	63%	13.6
9	1.3	M	Congenital glottosubglottic stenosis	Incomplete	98% (III)	?	100%	13.8
10	17.6	M	Postintubation subglottic stenosis	Complete	50% (I)	15	75%	10.3
11	5.2	F	Congenital tracheal stenosis	Complete	50% (I)	35	63%	29.5
12	44.8	M	Postintubation subglottic stenosis	Incomplete	100% (IV)	?	100%	25.4
13	17.6	F	Postintubation subglottic stenosis	Incomplete	75% (II)	?	72%	18
14	0.3	F	Congenital glottosubglottic stenosis	Incomplete	96% (III)	?	91%	8.9
15	22.0	M	Congenital subglottic stenosis and compression due to double aortic arch	Complete	50% (I)	6	34%	4.6
16	1.7	F	Left subglottic angioma	Complete	90% (II)	10	78%	13.4
17	30.5	F	Congenital glottosubglottic stenosis	Incomplete	70% (I)	?	76%	10.5
18	24.0	F	Postintubation subglottic stenosis	Incomplete	95% (III)	?	87%	10.1

*According to Cotton[1] system.

findings are compared and discussed.

PATIENTS AND METHODS

This study included 18 children assessed for dyspnea in the pediatric otorhinolaryngology department of La Timone Children's Hospital in Marseille, France, between November 1997 and November 1999. There were 10 girls and 8 boys with a mean age of 14 months (range, 3 weeks to 3.5 years) at the time of the procedure. All patients underwent video-recorded operative endoscopy, spiral CT, and virtual endoscopy with measurement of the stenosis. The length of stenosis and degree of narrowing obtained by each technique were compared. In 5 cases, modeling of the extraluminal lesion was also performed.

Operative Endoscopy. Laryngotracheal endoscopy was done under general anesthesia with spontaneous ventilation and direct viewing telescopes ranging from 2.7 to 4 mm in diameter and corresponding rigid bronchoscopes depending on the age and weight of the patient and on the degree of stenosis. The procedures were archived on videotape. The length of stenosis and degree of narrowing were evaluated visually by the endoscopist. Endoscopy allowed determination of the diagnosis, location, and approximate degree of narrowing in all cases. The cause of stenosis was subglottic angioma in 5 children and laryngotracheal stenosis in 13 (see Table). Stenosis was classified according to the 4-stage classification proposed by Cotton[1] as follows: grade I, less than 70% obstruction; grade II, between 70% and 90%; grade III, between 90% and 99%; and grade IV, 100%. Virtual endoscopy based on geometric modeling was archived on videotape.

Acquisition of CT Data. Axial spiral CT was performed on an Elscint CT-Twin scanner with a 180-mm acquisition field, a 512 × 512 reconstruction matrix, and 0.35-mm pixel size. The slice thickness was 1.3 mm with an interval of 1 mm (creating an overlap of 0.3 mm with each adjacent section). The pitch factor was 1. Images were formatted with the DICOM standard and then transferred to a Silicon Graphics 02-R10000 workstation.

Three-Dimensional Modeling. Three-dimensional modeling was based on a surface rendering technique developed for geometric modeling of abdominal aortic aneurysms from CT data.[11] This technique has been validated with phantoms and animal models. The first attempts at applying this modeling technique to the airways were made in 1998.[12]

The first step in reconstruction is selection of the area of interest (segmentation) on CT images to obtain a set of points on the boundary of the structure to be modeled. Segmentation is based on the principle of elastic matching[13] (Fig 1). The operator chooses the starting and ending cross-sectional CT images. After initializing the free-form contour on the start-

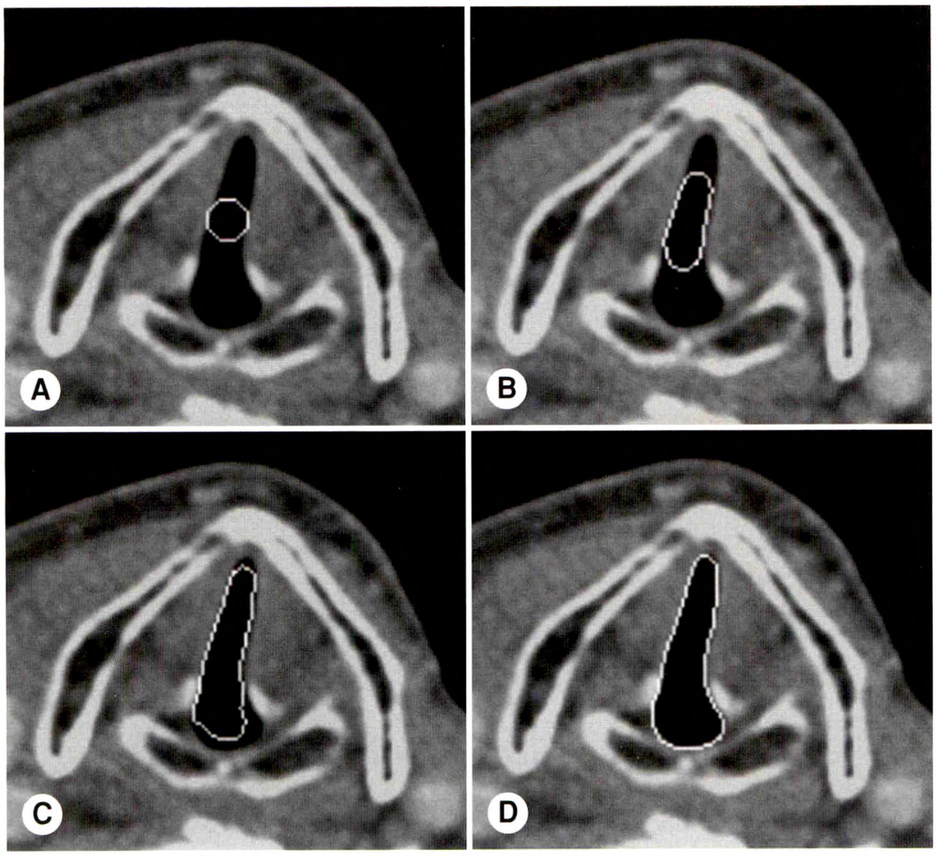


Fig 1. Detection of contour of airway lumen on cross-sectional computed tomography (CT) image by "elastic matching." **A)** Initialization. **B,C)** Expansion (snaking). **D)** Final contour.

ing image, the software computes the overall shape of the tubular structure based on projection of contours on successive slices. The operator can control the smoothness of the curve and the fitting process, which depend on the intensity, gradient, and texture of the image.[14]

After segmentation data are obtained, the 3-D parameters used for data acquisition are reintroduced to obtain a 3-D surface set of points. Modeling is based on the principle of the generalized cylinder, a mathematical tool that describes tubular shapes by means of an axis and sections perpendicular to that axis.[15] Data points obtained by image processing (Fig 2A) are reorganized into sections perpendicular to the axis. The tube is then generated as a free-form surface: a bicubic uniform B-spline, presenting properties of continuity at each point, adapting to the sections (Fig 2B). The axis is a fundamental component of this modeling technique, since it serves as the reference for calculation. The model can then be visualized in the wire-frame mode (Fig 3A) or the shaded surface display mode (Fig 3B). Specific tools have been developed, such as cutaway viewing (Fig 3C) and computer-generated endoscopy along the axis (Fig 3D).

Measurements. Tools have been designed to measure the length and degree of narrowing. To measure the length of a stenotic segment, the software calculates the distance S_1S_2 between the two points S_1 and S_2 selected on the axis by the operator. To determine the degree of stenosis, the operator selects a reference section (S_r) in a healthy zone and the section in which stenosis is to be measured (S_p). The area of each section is then calculated (A_r and A_p). The degree of narrowing is defined as the ratio between the stenotic and healthy areas (A_p/A_r) expressed in percent (Fig 4B).

Diameters (maximum, minimum, and mean) can be calculated in relation to the center of a given section. Since reconstruction is based on mathematical modeling, the process can be automatically extended along the entire axis to determine the profile of the tubular structure. Histograms showing the successive diameters of each section offer a visual profile of the diameter of the laryngotracheal lumen along the axis (Fig 4C).

Visualization of Extraluminal Involvement. Modeling of the laryngotracheal lumen alone does not allow visualization of the relationship between the lesion and surrounding structures. This may be done by overlaying CT images on the model to locate the lumen with respect to the other cervical structures. The CT images to be displayed are selected by the operator, who positions the corresponding sections along the axis (Fig 5A,B). Geometric modeling of extraluminal lesions was performed in the 5 cases of

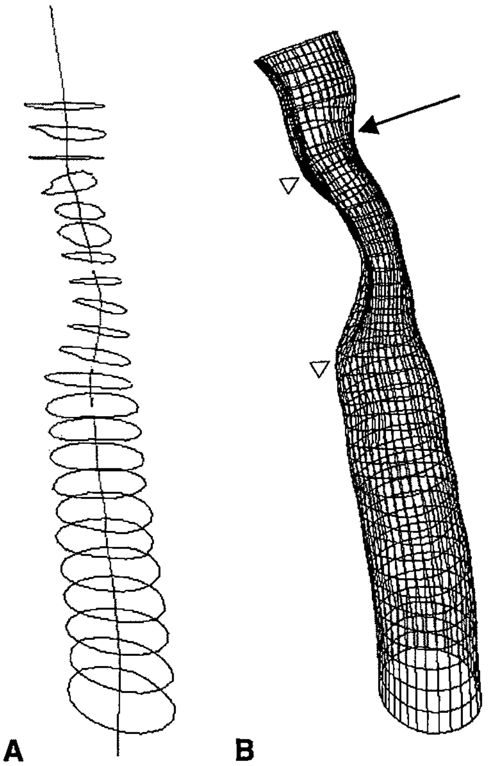


Fig 2. Calculation of model and visualization of surface. **A)** Visualization of central axis of sections calculated perpendicular to axis. **B)** Wire-frame representation: posterior view (left lateral stenosis). Arrow indicates level of glottis. White triangles show limits of narrowing.

subglottic angioma included in this study. Reconstruction was achieved by the same techniques used for the airways, ie, segmentation by elastic matching followed by surface modeling.

RESULTS

Operative endoscopy under general anesthesia demonstrated grade I stenosis in 8 cases, grade II stenosis in 5 cases, grade III stenosis in 4 cases, and grade IV stenosis in 1 case. In 9 cases (50%), the endoscopist was able to pass through the stenosis and achieve complete evaluation including estimation of the length of the lesion (see Table). In the remaining 9 cases, passage through the stenosis was prevented by either high-grade stenosis or the risk of respiratory compromise. The length of stenosis was not estimated in these cases.

Virtual endoscopy allowed measurement of the degree and length of stenosis in all 18 cases (see Table). In 10 cases (55%), measurements obtained by geometric modeling confirmed the degree of stenosis as estimated by the endoscopist. In 3 cases (17%), modeling underestimated the degree of stenosis. Two of these underestimated cases involved children with subglottic angioma (cases 2 and 16) that regressed under corticosteroid treatment in the interval between operative endoscopy and spiral CT scanning. In the third case (case 15), underestimation was due to a partial volume artifact on CT images that led to undersizing of the stenosis during modeling. In 5 cases (28%), modeling overestimated the degree of stenosis. In 2 cases (11%) involving grade III stenosis (cases 7 and 9), the residual airway was too narrow to be detected by CT. In 1 case (6%), secretions produced during CT scanning concealed the residual airway (case 10). In 2 cases (11%), modeling demonstrated that stenosis had been underestimated during operative endoscopy (cases 8 and 11).

Geometric modeling allowed measurement of the length of the stenosis in all cases. In the 9 children in whom complete operative endoscopy was achieved, modeling confirmed the endoscopist's findings. In the 9 children in whom complete operative endoscopy was not achieved, modeling allowed calculation of the length of stenosis. In 3 of these cases (cases 7, 9, and 12), the laryngeal lumen was invisible on cross-sectional CT images. As a result, the junction between the proximal and distal ends of the stenosis had to be recreated in order to allow calculation of the length of stenosis (Fig 6).

Reconstruction and visualization of extraluminal involvement was successful in all 5 patients with subglottic angiomas. In the case shown in Fig 7 (patient 1), the angioma was located in the left posterolateral region. Figure 7A shows contrast enhancement of the angioma, and Fig 7B shows the result of surface modeling. Operative endoscopic findings (Fig 7C) were concordant with virtual endoscopic findings (Fig 7D). The wire-frame mode allowed "see-through" visualization of the extent of the angioma and its relationship with the neighboring structures (Fig 7E).

DISCUSSION

The elastic matching technique described in this report was an efficient tool for segmentation of cross-sectional CT images. Detection of homogeneous areas such as the laryngotracheal lumen was excellent. Since the deformable contours method is based on mathematical calculation, shape recognition provides precise segmentation of a specific location. Ultimately, the use of texture criteria should allow segmentation of more complex structures, such as laryngeal cartilage. Successful modeling of subglottic angioma constitutes a step toward this goal. In the future, this method will be extended to other cervicothoracic structures, such as the esophagus and large cervicothoracic vessels.

A major advantage of our technique of geometric modeling is accurate measurement. To our knowl-

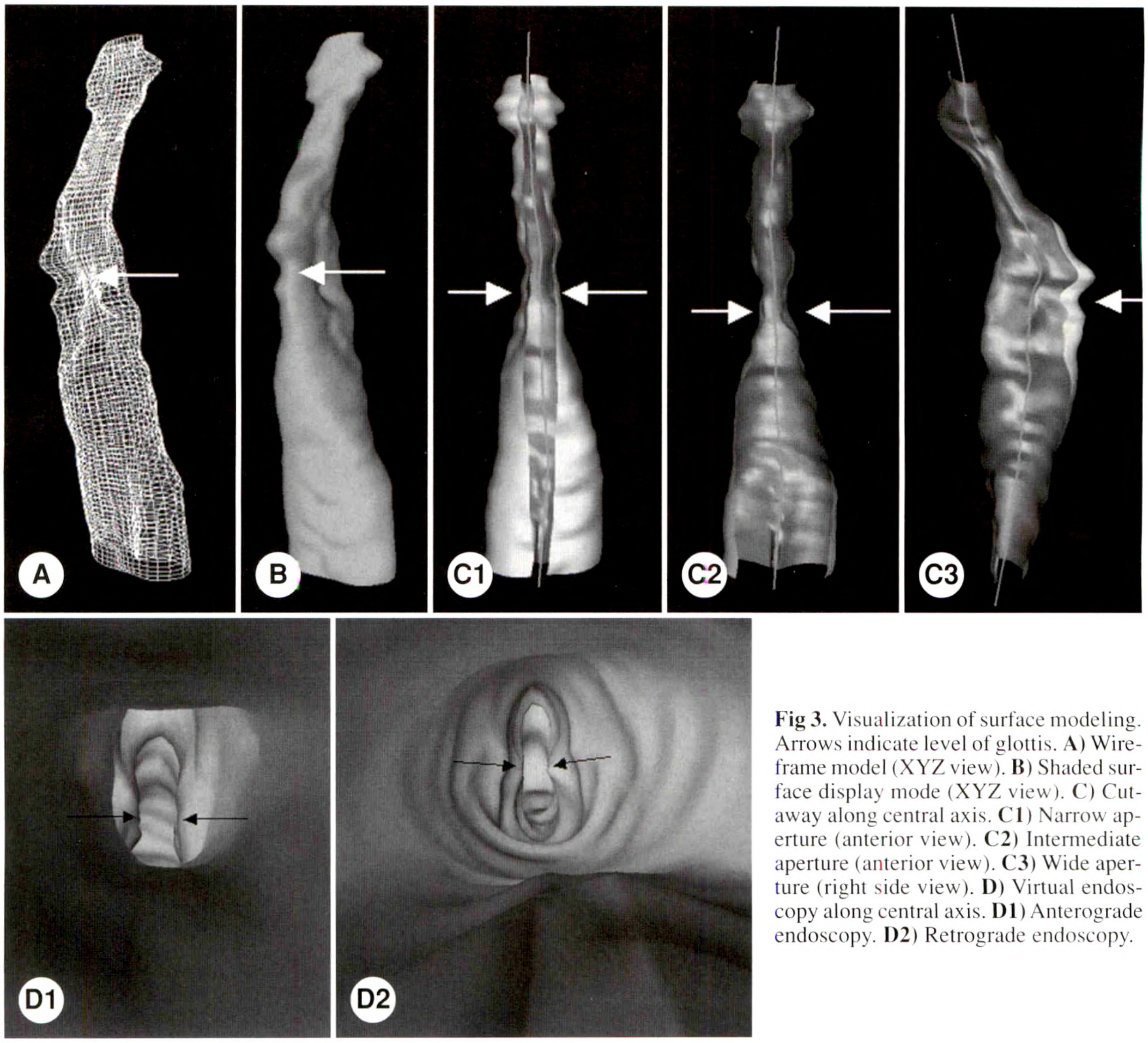
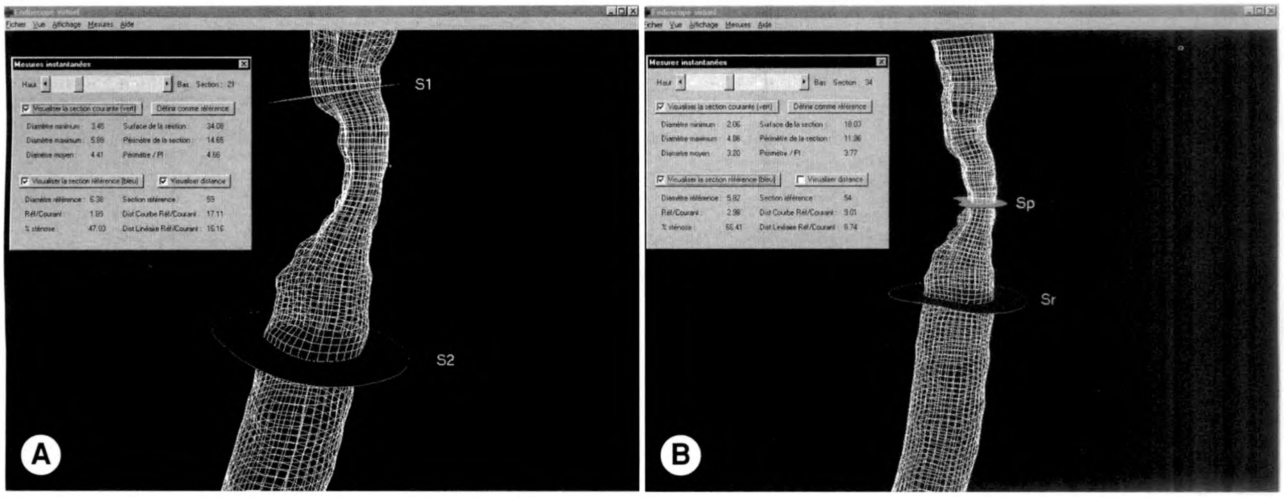


Fig 3. Visualization of surface modeling. Arrows indicate level of glottis. **A)** Wire-frame model (XYZ view). **B)** Shaded surface display mode (XYZ view). **C)** Cut-away along central axis. **C1)** Narrow aperture (anterior view). **C2)** Intermediate aperture (anterior view). **C3)** Wide aperture (right side view). **D)** Virtual endoscopy along central axis. **D1)** Anterograde endoscopy. **D2)** Retrograde endoscopy.

edge, the degree of accuracy achieved in this study cannot be reached with the 3-D imaging software now available on current imaging systems. The first reason is that axial native slices cannot allow diameter measurements perpendicular to the axis. The second is that 3-D volume reformatting such as multiplanar reconstruction provides such interactive measurements, but only at a few points, and not all along a curve axis.

Another advantage of this technique is interactive navigation. The operator can change the direction of visualization at any time. This is a major difference in comparison with real-time rigid endoscopy, in which viewing is limited to a single direction. Since this technique is based on mathematical calculation, the virtual endoscope can be used in the anterograde and retrograde directions (Fig 3D). Provided that the lumen is still visible on the corresponding cross-sectional CT image, the virtual endoscope can pass through a tight stenosis that would prevent passage of an operative endoscope.

Because modeling is mathematical, this technique facilitates development of powerful tools for measuring the degree and length of stenosis. The method used to determine the degree of stenosis in this study is relative to a selected reference section. This is the method used by most otolaryngological and vascular imaging systems. However, our modeling technique should enable absolute measurements by means of normalized tracheal size tables. Virtual endoscopy can also be useful for determining the length of stenosis in cases in which clinical evaluation is difficult or passage of a small-diameter endoscope is impossible. Measurement of diameter and length is important for therapeutic planning. Other advantages of surface reconstruction include shorter image processing times and see-through visualization in wire-frame or transparency mode. Three-dimensional navigation with this technique allows real-time interaction and nonlinear trajectories, so that the virtual endoscope


Endoscope virtuel
Fichier Vue Affichage Mesures Aide
Mesures instantanées
Haut
Bas
☑ Visualiser la section courante (vert)
Définir comme référence
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Définir comme référence
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☐ Visualiser distance
Sp
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Section : 21

Diamètre minimum : 3.45

Diamètre maximum : 5.89

Diamètre moyen : 4.41

Surface de la section : 34.08

Périmètre de la section : 14.65

Périmètre / PI : 4.66

Diamètre référence : 6.38

Réf/Courant : 1.89

% sténose : 47.03

Section référence : 59

Dist Courbe Réf/Courant : 17.11

Dist Linéaire Réf/Courant : 15.16

Section : 34

Diamètre minimum : 2.06

Diamètre maximum : 4.86

Diamètre moyen : 3.20

Surface de la section : 18.03

Périmètre de la section : 11.36

Périmètre / PI : 3.77

Diamètre référence : 5.82

Réf/Courant : 2.98

% sténose : 55.41

Section référence : 54

Dist Courbe Réf/Courant : 9.01

Dist Linéaire Réf/Courant : 8.74

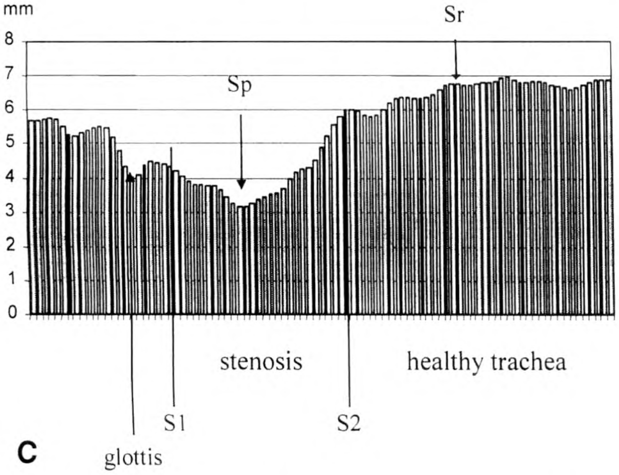

Fig 4. Measurement of diameter and length of stenosis. **A**) Measurement of length of stenosis by selection of 2 sections: S1 (proximal end of stenosis) and S2 (distal end of stenosis). **B**) Measurement of degree of stenosis by selection of reference section Sr (healthy tracheal) and pathological section (Sp) in stenotic zone. **C**) Histogram shows diameters (in millimeters) at different levels of laryngotracheal lumen. S1, S2, Sr, and Sp are indicated.

can be threaded through complex tortuosities that would prevent passage of an operative endoscope.

Because the quantity of data required for this mathematical surface method is relatively small, we have been able to develop software for desktop workstations (using the powerful OpenGL library) and to export geometric models over the Internet (using virtual reality modeling language technology). These operations cannot be done with current techniques based on volume-rendering reconstruction.[16]

In the future, use of virtual endoscopy in association with standard cross-sectional CT imaging and volume-rendering reconstruction could reduce the number of operative endoscopic procedures neces-

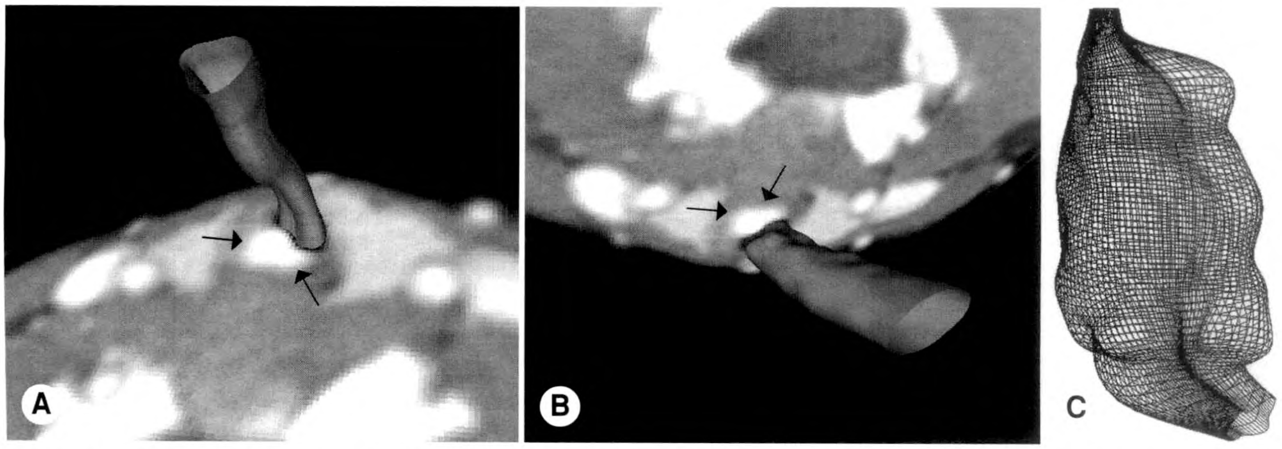

Fig 5. See-through visualization of extraluminal structures in wire-frame mode. Study of left posterolateral angioma. **A,B**) Projections of cross-sectional CT images in 3-dimensional (3-D) view. Arrows — subglottic angioma. **A**) Top view. **B**) Bottom view. **C**) Geometric modeling of angioma in wire-frame mode.

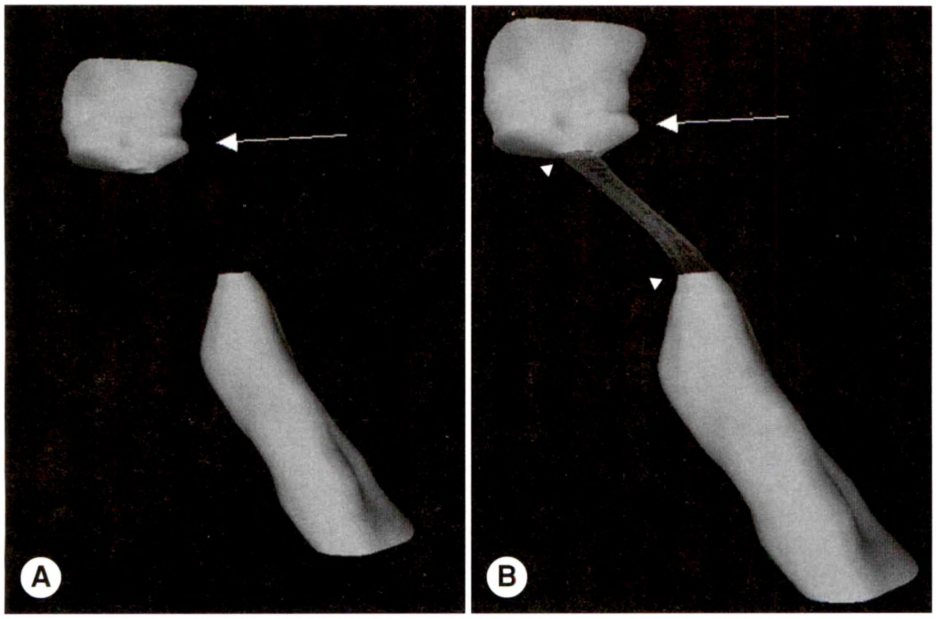

Fig 6. Model shows complete stenosis. Arrows indicate glottis. **A**) Visualization of segments above and below stenosis. **B**) Creation of junction to calculate length of stenosis (between triangles).

sary for diagnosis, therapeutic planning, and follow-up.

Our findings show that the delay between CT scanning and operative endoscopy should be as short as possible to ensure good correlation between the two procedures. Modeling and virtual endoscopy must be carried out under conditions comparable to those of therapeutic decision-making.

Virtual endoscopy provides useful diagnostic and therapeutic information, but imaging is a static geometric description of a dynamic reality. Features such as tissue texture (eg, elasticity) and wall movement

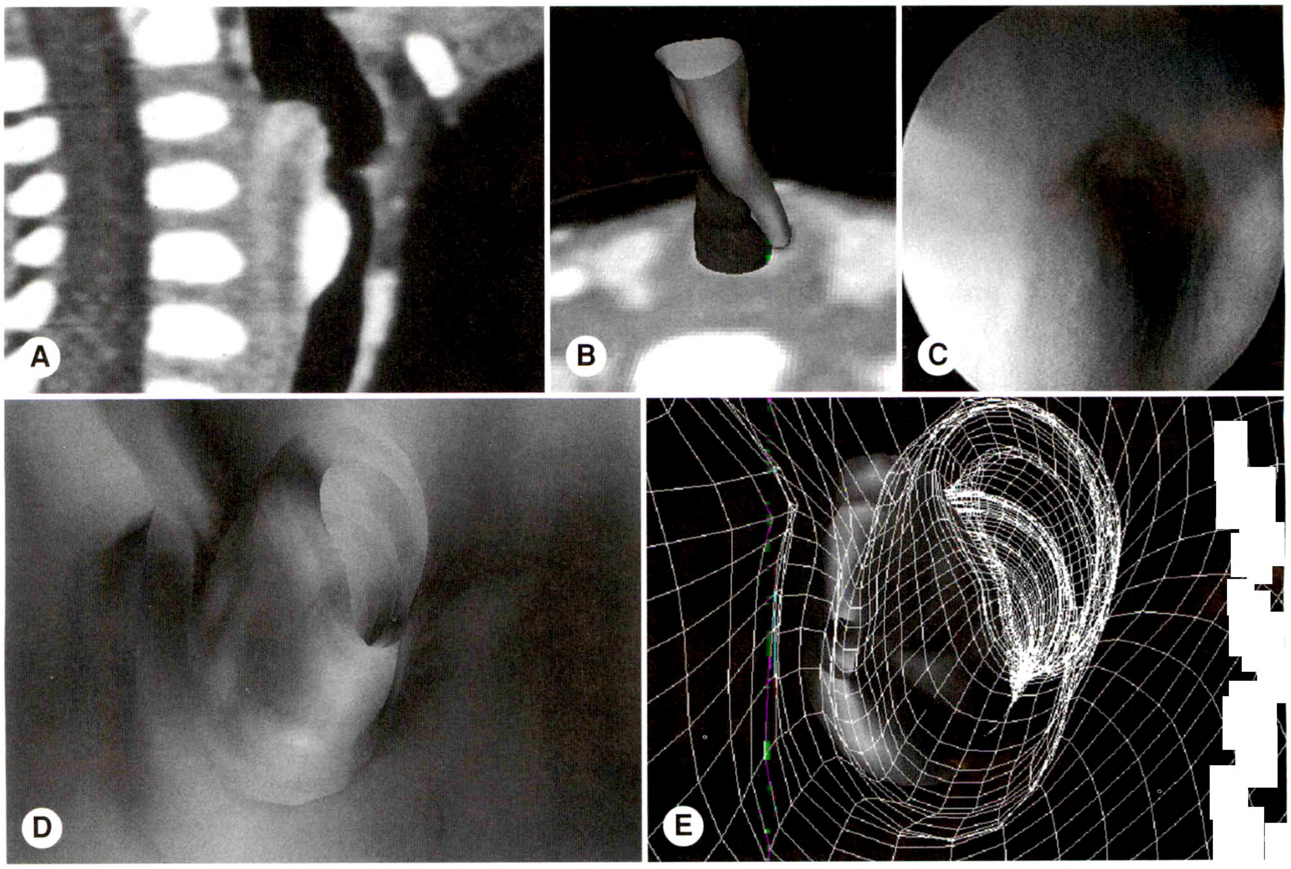

Fig 7. Modeling and study of subglottic angioma. **A**) Sagittal CT reconstruction. **B**) Modeling of angioma and 3-D view. **C**) Operative endoscopic view. **D**) Normal virtual endoscopic view. **E**) Virtual endoscopic view in wire-frame mode.

due to respiration and heartbeat are ignored.

CONCLUSION

The findings of this clinical research study document the potential utility of virtual endoscopy using surface modeling for evaluation of head and neck disorders. The results also reveal a few areas in which short-term improvements — like computational heuristics in 3-D modeling — are needed, notably with regard to methodology (segmentation of images) and applications (visualization of extraluminal structures such as cartilage, viscera, and tumor extension).

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EFFECT OF KETAMINE, DEXTROMETHORPHAN, AND MK-801 ON COCHLEAR DYSFUNCTION INDUCED BY TRANSIENT ISCHEMIA

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Overstimulation of the *N*-methyl-D-aspartate (NMDA) glutamate receptor has been implicated as a factor in the pathogenesis of hypoxic-ischemic injury in the central nervous system. To evaluate the role played by NMDA antagonists in ischemia-reperfusion injury of the cochlea, 3 noncompetitive NMDA antagonists — ketamine, dextromethorphan, and MK-801 — were administered to 53 albino guinea pigs subjected to transient ischemia of 30 minutes' duration, and the threshold shifts of the compound action potential were compared with those of nontreated animals 4 hours after the onset of recirculation. Ketamine and dextromethorphan moderately ameliorated the compound action potential threshold shifts, whereas MK-801, the most potent NMDA receptor antagonist among these 3 agents, did not show any protective effect. These results indicate that the action antagonizing the NMDA receptor has no protective effect against ischemia-reperfusion injury of the cochlea, and that ketamine and dextromethorphan act as protective agents for the cochlea via other pathways.

KEY WORDS — cochlea, compound action potential, ischemia, noncompetitive NMDA antagonist.

INTRODUCTION

It is well known that massively released glutamate, an excitatory neurotransmitter, has neurotoxic properties in the central nervous system, and that excessive activation of *N*-methyl-D-aspartate (NMDA) receptors by glutamate is associated with a wide range of neurologic disorders, including hypoxic-ischemic brain injury, trauma, epilepsy, Parkinson's disease, Huntington's disease, and acquired immunodeficiency syndrome dementia.[1] It has been reported that glutamate is a putative neurotransmitter at the synapse between the inner hair cells and the dendritic projections of the spiral ganglia in the cochlea,[2] and that ischemia induces acute swelling of the afferent dendrites on the basal side of the inner hair cells.[3,4] These results strongly indicate that excitotoxicity is also induced in the ischemic cochlea by excessive amounts of glutamate. Pujol et al[3] and Puel et al[4] showed that 6-7-dinitroquinoxaline-2,3-dione (DNQX), an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate antagonist, almost completely prevented ischemia-induced swelling of afferent dendrites, whereas no such effect was observed in animals treated with D-2-amino-5-phosphonopentanoate (D-AP5), an NMDA antagonist. They concluded from these results that NMDA receptor antagonists alone did not have any protective effect on cochlear ischemia. Their observations were, however, limited to histologic findings made during ischemia, and there was no information regarding the reperfusion period. Therefore, we were interested in examining whether animals treated with NMDA antagonists would have better physiological function of the cochlea after transient ischemia than would nontreated animals. The present study was undertaken to determine whether 3 noncompetitive NMDA antagonists — ketamine, dextromethorphan, and MK-801 — have any therapeutic effect on ischemia-reperfusion injury of the cochlea.

MATERIALS AND METHODS

Albino guinea pigs, weighing 300 to 400 g, with a normal Preyer pinna reflex, underwent tracheotomy under pentobarbital sodium anesthesia (28 mg/kg intraperitoneally) and artificial respiration. Catheters were placed in the right external jugular vein for drug administration and fluid replacement, and in the right carotid artery to monitor blood pressure. Rectal temperature was maintained at 37°C ± 1°C by a heat pack, and electrocardiogram and blood pressure were monitored throughout the experiment. The care and use of the animals reported in this study were approved by the Animal Experiment Committee at the University of Tsukuba.

Details of the methods used for measuring compound action potential (CAP) and inducing transient ischemia are described elsewhere.[5] Briefly, the left tympanic bulla was opened by the ventrolateral approach. A silver–silver chloride electrode was positioned at the bony edge of the round window of the

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cochlea, and the reference electrode was placed on the neck muscles. Tone bursts of 2- to 16-kHz pure tones (rise-decay time, 1 ms; duration, 10 ms; delivered every 200 ms in an open field system) were used to evoke the CAP, and 32, 64, or 128 consecutive potentials were averaged by a signal processor (Synax 2100, NEC Medical Systems, Tokyo, Japan). The sound pressure level (SPL) necessary to evoke a 10-µV CAP was defined as the threshold. When a sound of 110 dB SPL failed to evoke a potential of 10 µV, the threshold was defined as 115 dB SPL to simplify calculation.

The ventral surface of the brain stem was exposed by removing the bone of the skull base to visualize the anterior inferior cerebellar artery and the labyrinthine artery. Transient ischemia was induced by pressing the labyrinthine artery at the porus acusticus internus with a metal rod, and the blood flow was restored by releasing the pressure.[6] In order to monitor cochlear blood flow (CoBF), we used a laser-Doppler flowmeter (BRL-100, Bio Research Center, Nagoya, Japan). The needle probe was placed on the lateral wall of the second cochlear turn.

In order to evaluate the effects of ketamine, dextromethorphan, and MK-801 on the postischemic CAP threshold shifts, we subjected 53 animals to 30-minute ischemia. The CAP thresholds were measured before the administration of the drugs and 4 hours after the onset of reperfusion.

These animals were divided into the following 5 groups:

1. Ketamine-treated group ($n = 10$). Each animal was intravenously given 3 mg/kg ($n = 5$) or 10 mg/kg ($n = 5$) ketamine hydrochloride 4 times at 15-minute intervals starting 10 minutes before the onset of ischemia. Each infusion took 5 minutes to minimize the changes in arterial pressure.

2. Dextromethorphan-treated group ($n = 16$). Each animal received an intraperitoneal injection of 10 mg/kg ($n = 6$), 20 mg/kg ($n = 5$), or 30 mg/kg ($n = 5$) dextromethorphan hydrobromide 15 minutes before the onset of ischemia.

3. MK-801–treated group A ($n = 5$). Each animal received an intravenous injection of 0.5 mg/kg MK-801 30 minutes before the onset of ischemia.

4. MK-801–treated group B ($n = 12$). Artificial perilymph (137 mmol/L sodium chloride, 5 mmol/L potassium chloride, 2 mmol/L calcium chloride, 1 mmol/L magnesium chloride, 1 mmol/L sodium hydrogen carbonate, 11 mmol/L glucose, pH 7.4[7]) containing 1 µmol/L ($n = 4$), 10 µmol/L ($n = 4$), or 100 µmol/L ($n = 4$) MK-801 was perfused at a rate of 2.5 µL/min from 10 minutes before the onset of ischemia to 4 hours after the onset of reperfusion. The inlet hole was made in the scala tympani of the basal turn, and the outlet hole in the apex.

5. Nontreated control group ($n = 10$). Each animal was subjected to 30-minute ischemia, but received no further treatment.

To exclude the possibility that the ketamine, dextromethorphan, or MK-801 themselves might affect the CAP thresholds, we measured CAP before and 4 hours after administration of these drugs in 15 animals. The doses of ketamine, dextromethorphan, and MK-801 were 40 mg/kg (4 successive intravenous administrations of 10 mg/kg ketamine), 30 mg/kg given intraperitoneally, and 0.5 mg/kg given intravenously, respectively.

RESULTS

Figure 1 shows the results of laser-Doppler monitoring of CoBF before, during, and after transient ischemia. The CoBF was plotted as a percentage of the value before drug administration, and the postmortem value was set at 0%. Although the laser-Doppler flowmeter suggested the existence of a small amount of residual blood flow during ischemia, a previous study had determined that this response does not imply the presence of blood flow within the cochlea, but reflects a spurious contribution from superficial layers such as the bony wall.[5] When the artery was unclamped, the CoBF promptly increased with a transient overshoot and then gradually regained a stable level, close to the pre-ischemic value. Ketamine, dextromethorphan, and MK-801 did not significantly affect the CoBF (1-way analysis of variance [ANOVA]: $p > .05$).

Administration of ketamine (4 successive intravenous administrations of 10 mg/kg ketamine), dextromethorphan (30 mg/kg given intraperitoneally), or MK-801 (0.5 mg/kg given intravenously) did not affect the CAP threshold in control animals ($n = 5$ in each group; 2-way ANOVA: $p > .05$; data not shown).

Figure 2 shows the effects of ketamine, dextromethorphan, and MK-801 on the ischemia-induced CAP threshold shifts. Four successive intravenous administrations of 10 mg/kg ketamine significantly improved the CAP threshold shifts as compared with the nontreated control group (2-way ANOVA: $F = 13.9$, $p < .01$; Scheffe test: $p < .01$), whereas 4 administrations of 3 mg/kg ketamine did not show any protective effect (Scheffe test: $p > .05$; Fig 2A).

Intraperitoneal administration of 20 or 30 mg/kg of dextromethorphan significantly improved the CAP

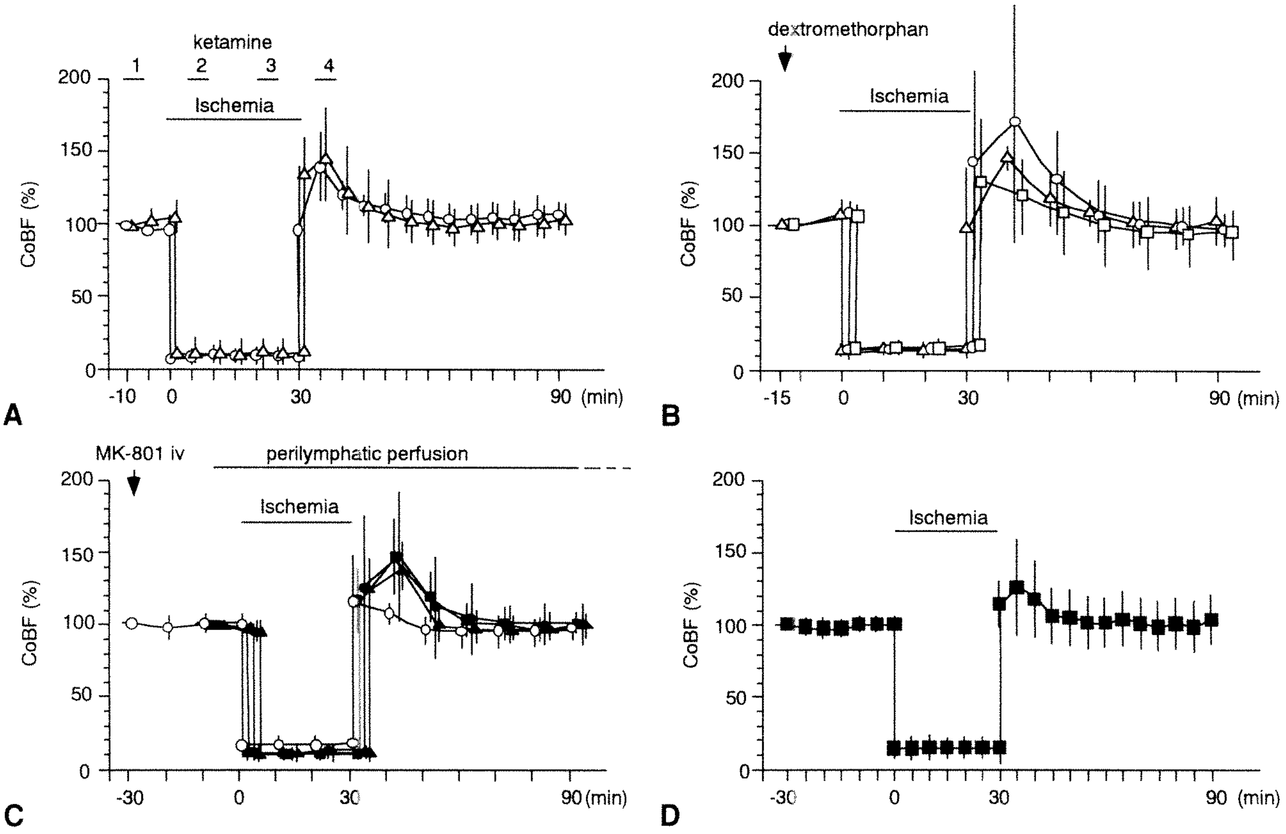

Fig 1. Cochlear blood flow (CoBF; percent before, during, and after onset of ischemia; mean ± SD). **A)** Ketamine-treated group. Circles and triangles indicate 10- and 3-mg/kg subgroups, respectively. Horizontal lines 1, 2, 3, and 4 indicate ketamine administrations. **B)** Dextromethorphan-treated group. Triangles, circles, and squares indicate 30-, 20-, and 10-mg/kg subgroups, respectively. **C)** MK-801–treated group. Open circles indicate group treated intravenously, with 0.5 mg/kg (MK-801–treated group A). Squares, closed circles, and triangles indicate 100-, 10-, and 1-μmol/L subgroups, respectively (MK-801–treated group B). **D)** Nontreated control group.

threshold shifts (2-way ANOVA: F = 10.3, p < .01; Scheffe test: p < .01 in the 30-mg/kg subgroup and p < .05 in the 20-mg/kg subgroup), whereas no improvement was observed after 10-mg/kg administration (Scheffe test: p > .05; Fig 2B).

Intravenous administration of 0.5 mg/kg of MK-801 did not show any protective effect (Fig 2C; 2-way ANOVA: F = 0.41, p > .05). In addition, perilymphatic perfusion of 1 to 100 μmol/L MK-801 failed to attenuate the postischemic CAP threshold shift (Fig 2D; 2-way ANOVA: F = 0.54, p > .05).

DISCUSSION

Since there were no available data on the cochlea at the time the study began, the protocols used to administer ketamine, dextromethorphan, and MK-801 in the present study were based on the results of rodent models of central nervous system ischemia. It has been shown that intravenous administration of ketamine (more than 1 mg/kg) reduces excitation of the rat spinal cord neurons caused by NMDA, but that this antagonism disappears in 10 to 120 minutes, depending on its dose.[8] Furthermore, it has been reported that the repeated intravenous administration of ketamine starting 10 minutes before the onset of ischemia decreases brain ischemic injury (dose range, 5 to 10 mg/kg).[9] For these reasons, we selected the administration method of ketamine used in the present study. A similar reduction in excitation of the rat spinal cord neuron induced by NMDA was seen after intravenous administration of 0.1 to 0.5 mg/kg MK-801, and this effect was long-lasting (more than 4 hours).[10] It has been shown that MK-801 intravenously administered 15 or 30 minutes before ischemia (dose range, 0.25 to 0.5 mg/kg) protects the brain from ischemic injury.[9,11] Furthermore, 0.01 to 1 μmol/L MK-801 blocks the response of cortical neurons to NMDA.[10] Thus, it is highly likely that the present administration methods, ie, a single intravenous injection of 0.5 mg/kg MK-801 or continuous perilymphatic perfusion of 1 to 100 μmol/L MK-801, provide substantial NMDA antagonism. In the case of dextromethorphan, the degree and time course of NMDA antagonism following its administration are not fully known. However, dextromethorphan administered within 30 minutes before induction of ischemia has been reported to exhibit protective effects

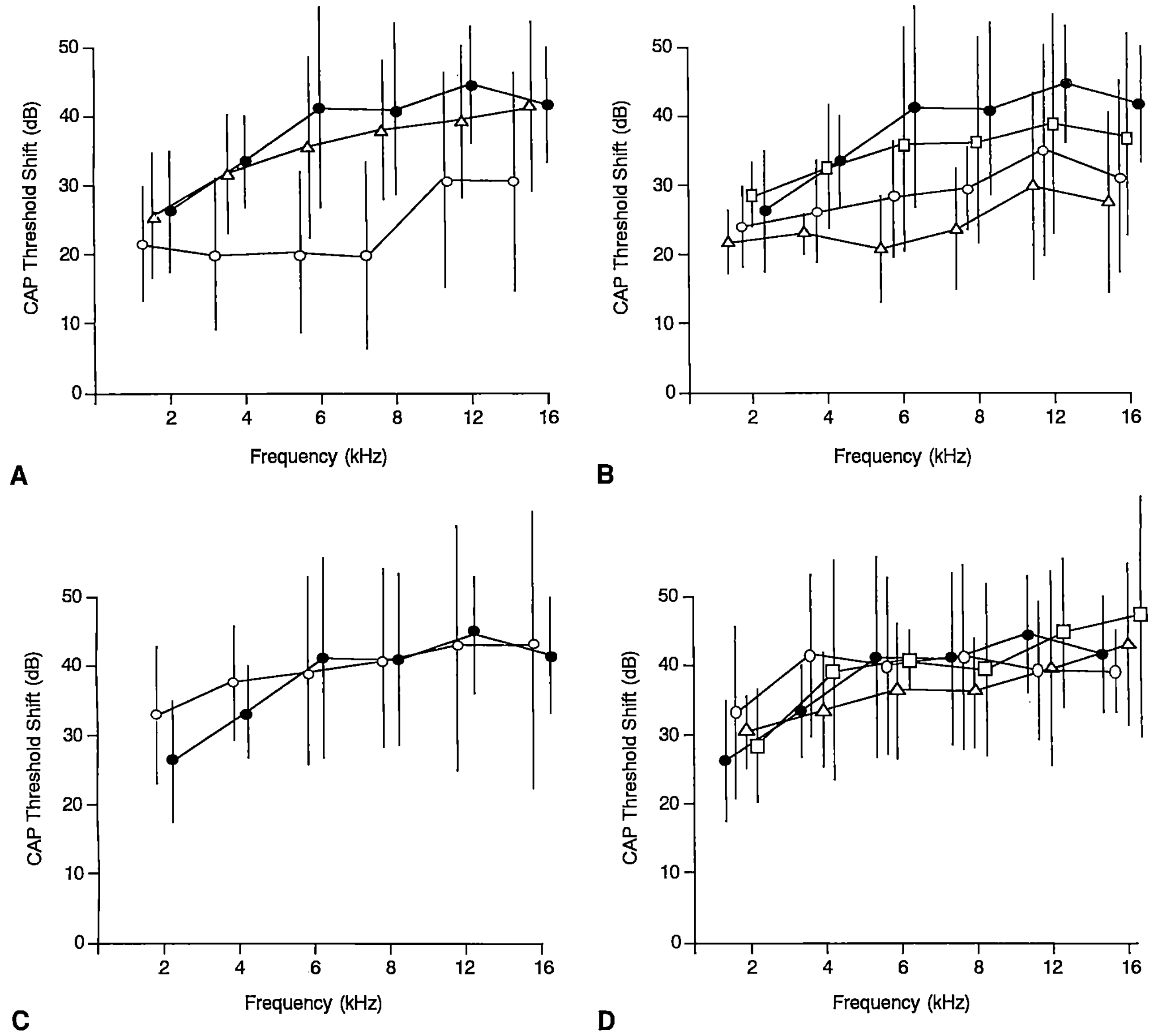

Fig 2. Compound action potential (CAP) threshold shifts from pre-ischemic values. Mean values ± SD at 4 hours after restoration of cochlear blood circulation are plotted. **A**) Ketamine-treated group. Open circles, triangles, and filled circles indicate 10-mg/kg (n = 5) and 3-mg/kg (n = 5) subgroups and control group (n = 10), respectively. **B**) Dextromethorphan-treated group. Triangles, open circles, squares, and filled circles indicate 30-mg/kg (n = 5), 20-mg/kg (n = 5), and 10-mg/kg (n = 6) subgroups and control group (n = 10), respectively. **C**) MK-801–treated group A. Open circles and filled circles indicate MK-801–treated group (n = 5) and control group (n = 10), respectively. **D**) MK-801–treated group B. Open circles, triangles, squares, and filled circles indicate 100-μmol/L (n = 4), 10-μmol/L (n = 4), and 1-μmol/L (n = 4) subgroups and control group (n = 10), respectively.

on ischemic brain injury.[12,13]

Although there is largely consensus that NMDA antagonists reduce the infarction volume in animal models of focal brain ischemia, several recent studies have questioned their effectiveness in models of global brain ischemia. Buchan and Pulsinelli[14] and Buchan et al[15] reported that a wide dose range of MK-801 failed to prevent hippocampal damage in a modified 4-vessel occlusion model of forebrain ischemia, and that the previously reported protective effect of MK-801 on ischemic hippocampal damage seemed to be related to hypothermia induced by MK-801. Our review of the available literature provided no obvious explanation for the different effects of NMDA receptor antagonists on focal versus global brain ischemia. In contrast to the controversy about the effect of MK-801, both dextromethorphan[12] and ketamine[16] were proved to be effective in models of global brain ischemia under precise temperature control. These results may reflect the need for thorough analysis as to whether the protective effect of ketamine and dextromethorphan is really due to inhibition of NMDA receptors.

The presence of NMDA receptors in the cochlea has been confirmed by in situ hybridization and immunohistochemistry, although they are believed to play only a minor role in normal auditory transmission.[17,18] It has been reported that spiral ganglion cells express messenger RNAs of the NMDA receptors NR1, NR2B, and NR2D at moderate to high levels.[18] The neurotoxic effect of NMDA and glutamate on cultured rat spiral ganglion neurons has been shown to be suppressed by an NMDA antagonist, D-AP5.[19] In in vivo studies, MK-801 protected against severe hypoxia (carbon monoxide–induced hearing loss),[20] but D-AP5 had no protective effect on the ischemia-induced swelling of the radial dendrites.[3,4] Unquestionably, there are some differences in the cellular mechanisms involved in these different ischemia and hypoxia models, reflecting, in part, differences in the depth of ischemia and the presence or absence of subsequent reperfusion.

In the present study, by pressing and releasing the labyrinthine artery, we induced ischemia and reperfusion. Since MK-801, the most potent NMDA antagonist among the 3 agents examined in the present study,[21] failed to attenuate the postischemic threshold shifts, we concluded that NMDA antagonists do not have any protective effect on cochlear ischemia-reperfusion injury, and this result is consistent with the morphological findings observed during ischemia by Pujol et al[3] and Puel et al.[4] In contrast to the negative effect of MK-801, ketamine and dextromethorphan ameliorated the postischemic CAP threshold shift. It is highly likely that these agents act via a pathway(s) other than NMDA-receptor inhibition.

Ketamine has antagonized nitric oxide release from the cerebral cortex after middle cerebral artery ligation,[22] and has also increased dopamine release in the rat medial prefrontal cortex.[23] Dextromethorphan and its metabolite, dextrorphan, have decreased potassium depolarization–evoked calcium uptake into brain synaptosomes and cultured neural cells.[24] Since a nitric oxide synthase inhibitor,[25] a dopamine agonist,[26] and calcium antagonists[27] have been reported to exert protective effects in cochlear ischemia, the protective effects observed in the present study may be explained, at least in part, by the involvement of these mechanisms (ie, blockade of nitric oxide release and increase in dopamine release caused by ketamine, and decrease in calcium uptake caused by dextromethorphan).

Some studies have suggested that NMDA receptors are important in repair of the inner hair cell–auditory nerve synapses.[28,29] It has been shown that synaptic damage from AMPA or kainate begins to heal some hours after an episode of excitotoxicity and has completely recovered within several days.[28-30] Upregulation of NMDA receptors has been demonstrated by an in situ hybridization method during this recovery period.[29] Furthermore, D-AP5 has been shown to delay the recovery from AMPA excitotoxicity.[28] Because of the limited observation period, we were unable to determine in the present study whether similar synaptic repair would occur after transient ischemia and whether NMDA receptors would be involved in this synaptic repair. It is necessary to develop a new model of cochlear ischemia to examine synaptic repair processes following ischemia.

In conclusion, 1) MK-801 did not affect the CAP threshold shifts following ischemia, and 2) ketamine and dextromethorphan showed protective effects on ischemia-reperfusion injury of the cochlea, although the protection mechanism remains undefined. From these results, we concluded that NMDA receptor antagonism alone did not protect the cochlea from this injury.

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NEAR-TOTAL LARYNGECTOMY IN ADVANCED CANCERS OF THE LARYNX AND PYRIFORM SINUS: A COMPARATIVE STUDY OF MORBIDITY AND FUNCTIONAL AND ONCOLOGICAL OUTCOMES

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This prospective study, performed from 1991 to 1996, analyzes the differences in oncological safety, functional utility, and surgical morbidity in 14 advanced lesions of the larynx (10 T3 and 4 T4; 7 N+) and 40 pyriform sinus lesions (1 T2, 20 T3, and 19 T4; 29 N+) subjected to Pearson near-total laryngectomy. The laryngeal cancer patients healed much faster, with a minimal wound complication rate of 28%, in comparison to the 68% rate encountered in the pyriform sinus cases ($p < .05$). The 3-year disease-free survival rate for the laryngeal cancers was 74%, while the 5-year survival rates for pyriform sinus cases were 66% for medial wall lesions and 54% for lateral wall lesions. Lung-powered shunt speech deemed qualitatively superior by acoustic analysis was obtained in 81% of the individuals (93% in laryngeal cases and 76% in pyriform sinus cases). Aspiration-free deglutition was achieved by 90% over periods ranging from 15 to 30 days. This study conclusively attests to the therapeutic efficacy of near-total laryngectomy for advanced lesions of the larynx and pyriform sinus that are unsuitable for radiotherapy, that are deemed too large or risky (because of aspiration) for partial laryngectomy, and that in the past would have merited total laryngectomy.

KEY WORDS — cancer of larynx, cancer of pyriform fossa, disease control, functional outcome, morbidity, near-total laryngectomy.

INTRODUCTION

Despite satisfactory local control rates[1,2] with wide-field laryngectomy (with comprehensive neck dissection clearing levels 1 through 5 in the N+ neck) and adjuvant radiotherapy in advanced laryngeal and pyriform sinus cancers, less-than-satisfactory speech rehabilitation[3] has marred universal acceptance. The success of tracheoesophageal prostheses reported by Western authors[4] has been difficult to reproduce in India, owing to the low health awareness and logistical problems that impede safe and successful prosthesis maintenance. An advanced stage of disease at initial presentation does not permit the routine execution of partial laryngectomy; this problem is reflected by our hospital cancer registry data (Kidwai Memorial Institute of Oncology, 1992), which indicate that only 10% of hypopharyngeal cancers and 20.2% of laryngeal cancers belong to stages 1 and 2. It is therefore not surprising that the Department of Head and Neck Surgery at the Kidwai Memorial Institute of Oncology performed only 36 partial laryngectomies, in contrast to 342 ablative procedures, over the 10-year period 1985 to 1995. Pearson et al[5-7] described the near-total laryngectomy, which permits fashioning of an innervated myomucosal tube from the contralateral, uninvolved side of the larynx that acts as a conduit to transmit air from the trachea to the neopharynx when exhalation is carried out against an occluded tracheostoma. The speech thus produced is not associated with the troublesome tracheobronchial spillover that is often associated with extended partial laryngeal resections and has been alluded to as "surgical brinkmanship" by the proponents of near-total laryngectomy.[5-8] The only prerequisite for this procedure appears to be the presence of a tumor-free zone between the two arytenoid cartilages with the preservation of the contralateral side of the larynx and one arytenoid cartilage that has its sensory and motor innervation intact. The oncological safety has been comparable to that of total laryngectomy[5-9] and has certainly been better than that obtained after definitive radiotherapy in stage-matched historical controls at this center (unpublished observations).

This prospective study was undertaken at the Kidwai Memorial Institute of Oncology, Bangalore, India, where cancers of the larynx and hypopharynx

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constitute 5% and 13%, respectively, of the 1,672 new cancers of the upper aerodigestive tract encountered annually (cancer registry data, Kidwai Memorial Institute of Oncology, 1992). It addresses the practice of near-total laryngectomy with the following aims and objectives: 1) to study the feasibility and morbidity of near-total laryngectomy in terms of complications, hospital stay, and success of postoperative radiotherapy (if deemed necessary by adverse final histologic results) by performing a comparative analysis of the outcomes obtained when the procedure is applied to lesions of the larynx and pyriform sinus; 2) to assess disease control in patients with a median follow-up of 32 months (range, 3 to 98 months); and 3) to evaluate speech acquisition, perform an acoustic analysis of shunt speech, and perform a subjective evaluation of aerodynamic parameters and intelligibility, for a comparison between the rehabilitative outcomes of lesions of the larynx and pyriform sinus.

METHODS AND MATERIALS

Fifty-four advanced squamous cell carcinomas — 14 of the larynx (10 T3 and 4 T4; 7 N+), 27 of the medial wall of the pyriform sinus (1 T2, 16 T3, and 10 T4; 17 N+), and 13 of the lateral wall of the pyriform sinus (4 T3 and 9 T4; 12 N+) — were endoscopically evaluated under general anesthesia to ensure a tumor-free zone between the two arytenoid cartilages. The pyriform sinus lesions were subdivided into medial wall and lateral wall lesions according to the location of the bulk of the tumor. All lesions were deemed beyond the realm of the conventional or extended forms of partial functional laryngectomy. None were evaluated by computed tomography or magnetic resonance imaging. Fifty-two men and 2 women 24 to 72 years of age (mean, 48.5 years) underwent Pearson near-total laryngectomy[5-8] with the resection margins controlled by frozen section histopathology. Tissues from the superior, inferior, anterior, and posterior aspects of the mucosal-submucosal margins were sent for frozen section evaluation after tumor resection was achieved. The N0 necks were subjected to level 2 through 4 clearance on the ipsilateral side routinely, and on the contralateral side if intraoperative inspection and palpation revealed enlarged nodes. Clinically significant metastatic nodes were routinely subjected to comprehensive neck dissection clearing levels 1 through 5. All patients with lesions of the larynx except for 1 needed a pharyngeal cutback flap for fashioning a tracheopharyngeal speech shunt that could be tubed around a 14F catheter to yield a lumen caliber with a diameter of 6 mm. In the patients with pyriform sinus lesions, 1 pharynx needed to be augmented with a pectoralis major myocutaneous flap for reconstructing a neopharynx of adequate dimensions. Advanced disease was defined as a T3 or T4 lesion or a lesion in which the final histopathology confirmed microscopic tumor and/or severe dysplasia or carcinoma in situ at the specimen periphery or within 5 mm of the tumor edge, multiple metastatic nodal deposits with evidence of perinodal disease extension, or other adverse histologic findings such as perineural or perivascular disease. These lesions mandated external-beam tele-irradiation in all cases of pyriform sinus lesions and in 79% of such cases with laryngeal cancers. The teleradiotherapy was begun within 4 weeks in 39 individuals (10 laryngeal and 29 pyriform sinus lesions), with no postoperative complications and satisfactory wound healing. External teletherapy was delivered by a cobalt 60 source with a source-to-skin distance of 80 cm. The tumor bed and both sides of the neck received an average dose of 56 Gy over $5^1/_2$ weeks at 2 Gy per fraction (5 fractions per week) by parallel opposed anteroposterior-posteroanterior fields (4:1 loading with spinal cord shielding in posteroanterior field) in addition to right lateral and left lateral portals. Speech therapy was begun after complete wound healing with restoration of normal swallowing.

The ease of speech acquisition and the quality, intelligibility, fundamental frequency, and intensity of speech after near-total laryngectomy were assessed objectively in a speech laboratory. Aerodynamic parameters were recorded in a sound-treated room with high-fidelity recording equipment. Intelligibility was subjectively evaluated by trained but "blinded" observers who scored recorded recitations of phonetically balanced words. Individuals with excellent intelligibility had scores of over 75%, and a score of 30% to 75% was deemed satisfactory. Those in whom fewer than 30% of the words were intelligible were considered poor speakers. Follow-up of 52 patients (excluding 2 who died) ranged from 3 to 98 months, with a median follow-up of 32 months. Patients with lesions of the larynx had a maximum follow-up of 3 years; the disease-free survival data were analyzed for this period (Fig 1); those from the pyriform sinus group had a maximum follow-up of 5 years and have therefore been analyzed for that period (Fig 2).

RESULTS

Microscopic positive tumors on the periphery of the near-total laryngectomy specimen at final histopathology contradicted the initial frozen section report in 6 individuals (1 larynx, 3 medial wall, and 2 lateral wall lesions of the pyriform fossa sinus; Table 1). A major pharyngocutaneous fistula was found in

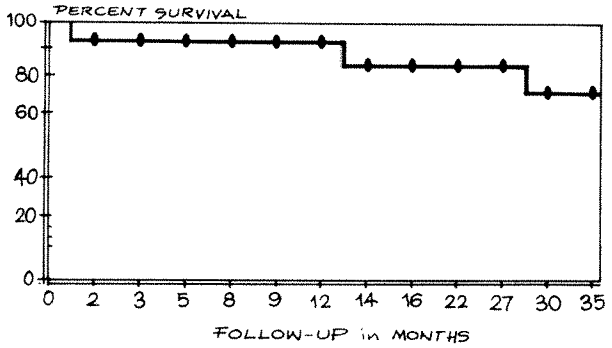

Fig 1. Kaplan-Meier survival curve for 14 patients with laryngeal cancer and near-total laryngectomy.

TABLE 1. FROZEN SECTION RESECTION VERSUS FINAL HISTOPATHOLOGIC DIAGNOSIS

	Laryngeal Lesions	*Medial Wall Pyriform Sinus Lesions*	*Lateral Wall Pyriform Sinus Lesions*
R0	13	24	11
R1	1	3	2
R2	2	2	2

R0 — No tumor was detected on microscopic examination of cut margins of specimen periphery at final histology.

R1 — Microscopic tumor was detected at cut margins of specimen periphery. Frozen section evaluation in these cases was negative, indicating satisfactory disease clearance, and was thus inaccurate in final analysis.

R2 — Gross disease was present that was not amenable to tumor-free resection by near-total laryngectomy technique. Hence, these 6 operations were converted to total laryngectomy during operation.

28% of patients with lesions of the larynx, as compared with 27% with medial wall and 33% with lateral wall lesions of the pyriform sinus (Table 2; not statistically significant). Complications that delayed convalescence were noted in 28% of patients with lesions of the larynx versus 68% of those with pyriform sinus lesions ($p < .05$). The median hospital stay was 22 days (range, 12 to 48 days). Those who developed wound complications had a mean hospital stay of 26 days (20 days for patients with lesions of the larynx versus 30 days for those with pyriform sinus lesions), in contrast to a range of 12 to 22 days for individuals who did not (Table 2). The subjective evaluation of shunt speech deemed the speech ability as excellent (N = 24) or satisfactory (N = 18) in 92% of patients with laryngeal cancers, as against 76% of patients with pyriform sinus lesions (not statistically significant). The mean time to maximum speech proficiency from the onset of speech therapy was, on average, 7 days for those rated as excellent, in comparison to 15 days for those whose speech was deemed satisfactory (Table 3). Acoustic analysis of recorded speech in a speech laboratory attested to a clear superiority of shunt speech over the speech of matched esophageal speakers also rated to have excellent communicative speech ability (Table 4). Ten individuals (19%) did not develop speech (Table 5): 8 because of shunt stenoses and 2 because of denervation of the shunt (based on electromyographic evidence).[10] None of these stenotic tracheopharyngeal speech shunts (7 pyriform sinus lesions and 1 laryngeal lesion) were amenable to remedial dilation with successful production of shunt speech. Symptomatic aspiration was documented among 5 patients who developed shunt speech (4 laryngeal lesions and 1 medial wall pyriform sinus lesion): 2 of these patients had minimal aspiration (defined as tracheal stain on milking the shunt), 2 patients had laryngeal lesions with moderate aspiration (defined as trickle into the shunt lumen of rapidly ingested liquids only with production of cough but no serious long-term pulmonary decompensation), and 1 patient (laryngeal lesion) had severe aspiration that culminated in lung abscess and subsequent fatal septicemia. All symptoms of moderate aspiration resolved within 4 weeks after the last fraction of radiotherapy. Fourteen laryngeal cancers, 28 medial wall pyriform sinus lesions, and 12 lateral wall pyriform sinus lesions have median follow-ups of 24.5, 46.5, and 20 months, respectively. The 3-year disease-free survival rate (Fig 1) determined by the Kaplan-Meier method for patients with laryngeal cancer was 74%. The 5-year disease-free survival rates for patients with pyriform sinus cancers were 66% for medial wall cancers and 54% for lateral wall cancers (Fig 2). The pattern of dis-

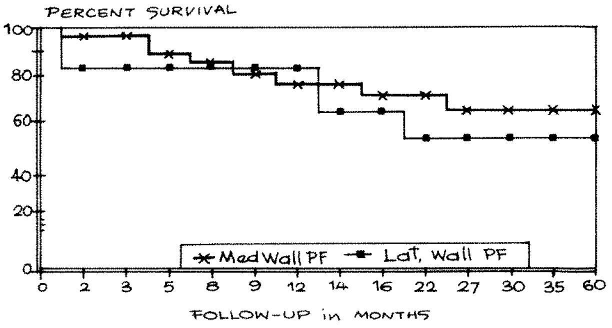

Fig 2. Kaplan-Meier survival curves for 27 patients with medial wall pyriform sinus (PF) cancer, and 13 patients with lateral wall PF cancer, and near-total laryngectomy.

TABLE 2. POSTOPERATIVE COURSE

	Laryngeal Lesions	*Medial Wall Pyriform Sinus Lesions*	*Lateral Wall Pyriform Sinus Lesions*
Fistula	4 (28%)	7 (27%)	4 (33%)
Infection	4 (28%)	10 (38%)	5 (42%)
Death	0	1 (4%)	1 (4%)
Dysphagia	0	1 (4%)	2 (16%)
Complication rate	4 (28%)	18 (66%)	7 (54%)
Hospital stay (d)	20 (16-28)* 15 (12-18)†	30 (20-48)* 18 (14-21)†	34 (21-46)* 17 (13-22)†

*With complications.

†Without complications.

TABLE 3. TIME TO ACQUISITION OF TRACHEOPHARYNGEAL SHUNT SPEECH

	Laryngeal Lesions	*Medial Wall Pyriform Sinus Lesions*	*Lateral Wall Pyriform Sinus Lesions*	*Mean (Range)*
Excellent (n = 24)	10	10	4	7 d (5-10 d)
Satisfactory (n = 18)	3	9	6	15 d (12-24 d)
Poor (n = 10)	1	7	2	0 (>3 wk)

TABLE 4. SHUNT PROBLEMS

	Laryngeal Lesions	*Medial Wall Pyriform Sinus Lesions*	*Lateral Wall Pyriform Sinus Lesions*
Shunt stenoses	1	5	2
Shunt denervation	0	2	0
Aspiration	4	1	0

ease recurrence was as follows: 2 of 14 patients with laryngeal cancers (1 bone metastasis and 1 stomal recurrence), 8 of 26 with medial wall pyriform sinus lesions (7 neck and 1 second primary in esophagus), and 4 of 12 with lateral wall lesions (1 neck metastasis, 1 neck metastasis with intracranial extension, and 2 deaths due to intercurrent disease). Two salvage radical neck dissections were successfully carried out for metachronous neck recurrence (with controlled primary), and these individuals have been included as disease-free survivors in the survival analysis.

DISCUSSION

One hundred two Pearson near-total laryngectomies were carried out for advanced lateralized lesions of the larynx (25) and pyriform sinus (77) at the Kidwai Memorial Institute of Oncology, Bangalore, from 1988 to 1998. This prospective study addresses the outcome of near-total laryngectomy in 54 consecutive advanced lesions of the larynx (14) or pyriform sinus (40) carried out by the senior author (A.M.S.) with the support of other members of the Department of Head and Neck Surgery over a 5-year period (1991 to 1995). An earlier report from this center attested to the therapeutic efficacy of this surgical procedure, with satisfactory speech restoration with no tracheobronchial aspiration.[8] Before 1988, these patients would have undergone total laryngectomy with intensive speech rehabilitation in the form of esophageal speech,[11] leading to a significant number of disease-free but dissatisfied individuals. Furthermore, the inability of our patient population to successfully adopt prosthetic options,[4] owing to their low level of health awareness and their prosthesis aftercare problems, has made their existence after total laryngectomy a difficult one and has contributed in no small measure to the poor acceptance of this procedure despite apparent oncological gains. The practice of functional partial resection is impeded in patients at our institution by the advanced stages of their lesions at initial presentation and is fraught with the dangers of troublesome tracheobronchial aspiration, which is directly related to the extent of resection and the patients' pulmonary reserve. Patients with these advanced lesions (according to historical stage-matched controls) also fare poorly with definitive radiotherapy; virtually no lesions were amenable to successful surgical salvage after control rates at 2 years of 13% in the pyriform sinus and 15% in the laryngeal group (unpublished observations). Poor control was largely attributed to an inability to carry out salvage surgery due to unreliable follow-up that resulted in a lesion's becoming unresectable or deterioration of the patient's general condition to a stage that was not conducive to successful surgical salvage.

The establishment of the Pearson near-total laryngectomy technique in the surgical repertoire has revolutionized the acceptance of primary surgery and led to decidedly better local control rates. The speech

TABLE 5. COMPARATIVE ANALYSIS OF SPEECH

Speech Parameters	*Near-Total Laryngectomy Speech (n = 11)*	*Esophageal Speech (n = 10)*	*Normal Indian Male*	*t-Test Value*
Average fundamental frequency (Hz)				
Mean	96.04	60.48	125.6	12.9518*
SD	4.58	7.75		
Average intensity (dB)				
Mean	70.30	56.48	78.9	4.346*
SD	4.80	9.74		
Maximum phonation duration (s)	8.70	1.80		
Syllables per breath	16.80	5.10		
Rate of speech (words per minute)	58.40	40.70		
Intelligibility (%)	84	77		

*$p < .01$.

advantage (in >80%), without troublesome tracheobronchial aspiration (<10%), has indeed made this procedure worth the marginally increased hospital stay. Attention is being directed to minimizing the local wound problems that mar the convalescence, especially in patients with pyriform sinus lesions. It appears that an increased predilection for local wound breakdown may be related not only to technical factors, such as a tight pharyngeal suture line, but to the underlying defects in cell-mediated immunity that are commonly responsible for increased postoperative infection after surgery for head and neck cancers.[12,13] Therefore, these procedures may be better avoided in clinical situations with a high potential for infection, such as radiotherapy failure and obstructive lesions in patients with prior tracheotomies.

For untreated advanced lateralized lesions of the larynx and pyriform sinus, this procedure has been practiced at our center without serious complications in more than 100 patients over a period of 10 years since the first near-total laryngectomy in 1988. Case selection based on the clinico-endoscopic profile led to successful execution of this procedure in 90% of the cases attempted, a rate akin to those in other reports.[14] While modern radiologic imaging techniques such as computed tomography and magnetic resonance imaging have a definite role in detecting subsurface spread, their routine use in this country may be difficult, owing to logistic and financial constraints. Frozen section–controlled resections supplemented the above-described clinico-endoscopic assessment to achieve a histologically complete resection in all except 6 lesions — mostly pyriform sinus lesions, which are notorious for their multicentricity and submucous skip lesions. The 6 specimens that showed microscopic positive or close margins did benefit from prompt institution of postoperative radiotherapy, for a gratifying locoregional control rate of 81% (44/54). No lesions with positive cut margins or close margins (tumor edge of <5 mm from specimen periphery) recurred in the neopharynx (pyriform sinus group) or the speech shunt (larynx group). Analysis of the locoregional recurrence pattern, which is the best yardstick to evaluate the efficacy of locoregional therapy, indicated neck recurrences almost exclusively among pyriform sinus cancers (9/10), with 1 stomal recurrence in a lone laryngeal case. This tendency reflects the biologically aggressive nature of pyriform sinus lesions, which was again confirmed by our Kaplan-Meier survival analysis, in which laryngeal cancers had a decidedly better outcome than pyriform sinus lesions — an observation that has been corroborated in stage-matched historical control patients with similar T3 and T4 lesions of the larynx and pyriform sinus who underwent total laryngectomy at our center (unpublished observations).

Functional outcome needs to be evaluated with respect to restoration of speech and normal swallowing. Speech was restored in 81% of patients within a period of 7 days of speech therapy in those deemed excellent speakers (n = 24) and within 15 days for those rated as satisfactory speakers (n = 18). This is in sharp contrast to Western reports, which quote periods ranging from 5.4 months[15] to 40 to 180 days[16] for attainment of maximum proficiency. Our experience indicates that if maximum proficiency is not attained by 4 weeks of daily speech therapy, the patient is unlikely ever to develop speech.[17] Successful speech development occurred more among patients with lesions of the larynx (92%) than among patients with medial wall or lateral wall pyriform sinus lesions (73% and 83%, respectively). A similar observation was made by Pearson[7] (90% in larynx versus 80% in pyriform sinus) and probably reflects the greater availability of laryngeal mucosa for tracheopharyngeal speech shunt reconstruction in comparison to the availability of pharyngeal mucosa for reconstruction of a spacious neopharynx. Among laryngeal lesions, it is the remnant mucosa (from the residual contralateral larynx) that needs to be augmented with a pharyngeal cutback flap (the adynamic component) to attain a diameter of 6 mm; after partial pharyngectomy for the pyriform sinus lesions, it is the pharyngeal mucosa that tends to be inadequate. Augmentation in either case needs to be done with caution; it may predispose to aspiration in laryngeal lesions if the resultant tracheopharyngeal shunt caliber is too large and may defeat the very objective of physiologic safety. In pyriform sinus lesions, augmentation of the pharyngeal lumen with a pectoralis major myocutaneous flap may lead to a neopharynx with inappropriate vibratory characteristics and poor speech rehabilitation. Stenosis, on the other hand, reflects an inadequate shunt lumen of a caliber not compatible with successful shunt valving and speech production. Stenosis often occurs if the shunt is unaugmented, as in 1 of the 14 laryngeal lesions in this study. A tight primary pharyngeal closure after excision of involved pyriform sinus tissue can exert extrinsic pressure and lead to an attenuation of a tracheopharyngeal shunt of adequate dimension (ie, 6-mm diameter). The sequela in either situation is unsatisfactory speech rehabilitation and a tract that is not amenable to periodic dilation with shunt valving. In Pearson's study[7] of 108 cases, 19 stenoses were noted, of which only 9 were successfully dilated. Krespi and Sisson[18] encountered more stenoses among their cases, which, incidentally, comprised exclusively pyriform sinus lesions. In addition, tight pharyngeal closure with available mucosa may predispose to wound breakdown and/or, in the long term, predispose to attenuation of the neopharynx with subsequent dysphagia. Unlike speech shunt stenoses, in our study attenuated neopharynges were amenable to dilation with improvement in swallowing. Suits et al[9] have drawn an association between pharyngocutaneous fistula and shunt aspiration (33%). Our study shows quite the contrary: tracheobronchial aspiration, albeit transient, was frequently encountered after near-total laryngectomy for laryngeal lesions, and postoperative recovery in these lesions was seldom associated with the local wound problems so commonly associated with near-total laryngectomy for pyriform sinus lesions. Shunt denervation was assumed to be the cause of speech failure in 2 individuals.[10] Fortunately, these denervated shunts did not leak or lead to uncontrolled aspiration, despite having a patent lumen as evidenced by free passage of a 14F catheter, as well as good pulmonary expiratory flow. Transcutaneous electromyographic studies[10] did not reveal any correlation between motor unit potentials measured at rest and those measured during activities such as deglutition or attempted speech in either speakers or nonspeakers. The Teflon electrodes implanted into the shunt wall elicited a resting tone potential that correlated with the videolaryngoscopic documentation of a closed shunt aperture at rest. During shunt valving with speech production, sporadic uncoordinated motor unit potentials with features suggestive of various degrees of denervation, such as motor unit dropout and incomplete recruitment, were noted. These electromyographic findings indicate that speech modulation is probably not a dynamic function; in all likelihood, successful speech generation may be related to airflow characteristics that evoke a reflex noncoordinated muscle activity. The anatomic design of the shunt, the location of its aperture favorably beneath the tongue base, and the resting muscle tone are probably the features that render the shunt aspiration-proof.

CONCLUSIONS

1. Lateralized T3 and T4 lesions of the larynx or pyriform sinus with a tumor-free zone between the two arytenoid cartilages as determined by clinical evaluation only, without sophisticated radiologic imaging, may successfully undergo frozen section–monitored near-total laryngectomy in more than 90% of cases.

2. When supplemented with adjuvant postoperative radiotherapy, this regimen gives disease control comparable to that of ablative procedures in stage- and site-matched historical controls. This finding has been supported by many reports.[7-10] Preliminary experience with chemoradiation organ preservation at this center has been impeded by poor compliance due to treatment-related costs and complications and has relegated this treatment strategy to clinical situations (chiefly, advanced lesions of the oropharynx or hypopharynx) in which surgical resection is associated with morbidity that prohibits restoration of normal swallowing (unpublished observations).

3. Marginally increased local wound problems complicate convalescence after near-total laryngectomy and lead to an increased hospital stay, especially in cases of pyriform sinus lesions. However, this initial disadvantage is compensated in the large majority of patients by aspiration-free swallow (90%) and superior speech (80%) at the final evaluation.

4. Symptomatic tracheobronchial aspiration has been noted in a small minority of cases (10%), predominantly laryngeal cancers, in which shunt augmentation was a necessity. Fortunately, in most cases, the aspiration subsided on its own over a period of time.

5. Shunt stenoses (15%) prevented successful valving of the tracheopharyngeal shunt, predominantly among pyriform sinus lesions and unaugmented laryngeal lesions. None of these stenosed shunts were rendered functional by repeated dilation.

6. Our finding of effortless acquisition of high-quality speech within a period of 2 weeks in 80% of individuals effectively abolishes "prosthesis dependence" on the part of physicians.

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BIOLOGY, PREVENTION, AND TREATMENT OF HEAD AND NECK CANCER

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SUBGLOTTIC STENOSIS COMPLICATED BY ALLERGIC ESOPHAGITIS

CASE REPORT

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Allergic esophagitis is a known entity that had been described in patients with dysphagia. It has not been previously described in association with subglottic stenosis. We report the case of a 2-year-old girl with symptoms suggestive of allergic esophagitis who suffered from subglottic stenosis that recurred despite surgical measures. Her esophageal pH monitoring results were normal, and she did not respond to antireflux medications. She did respond dramatically to corticosteroid therapy with improvement of both her esophageal and laryngeal symptoms. Allergic esophagitis as a clinical entity is discussed.

KEY WORDS — allergic esophagitis, subglottic stenosis.

INTRODUCTION

In 1982, Winter et al[1] described the association of esophageal eosinophilia with abnormal acid clearance. Since that point, esophageal eosinophilia has been used as a marker for gastroesophageal reflux disease (GERD). Gastroesophageal reflux disease has been linked to a host of airway disorders, including subglottic stenosis (SGS). The effect of GERD on the development and management of laryngotracheal stenosis was first described by Bain et al[2] in 1983 and has subsequently been the subject of much debate and controversy. In 1985, Little et al[3] performed an elegant experiment in which they examined the effect of gastric acid on injured laryngeal mucosa. In a canine model, they divided their experiment into two arms: a control arm and an experimental arm. Both groups of dogs underwent controlled endoscopic injury to the laryngeal mucosa; the subglottic mucosa of the dogs in the experimental arm was then painted with gastric acid. In the control animals, the mucosal injuries healed without the development of SGS. When the mucosal injury was painted with gastric acid, SGS developed. Koufman[4] has reported clinical data that concur with these canine experiments; he found that 78% of patients with SGS have abnormal double-probe pH study findings suggestive of GERD.

There have been several reports of patients with esophageal eosinophilia and the GERD-associated symptoms of dysphagia, vomiting, heartburn, and globus sensation who failed to respond to traditional medications for GERD such as prokinetics and proton pump inhibitors but who responded well to corticosteroid therapy.[5,6] This has given credence to the diagnosis of allergic esophagitis.[7,8] Kelly et al[9] demonstrated that in some cases food allergy produces eosinophilic esophagitis. Ten children with GERD-like symptoms failed to respond either to maximum medical therapy or to surgical Nissen fundoplication, but improved with dietary restrictions and a specific diet; symptoms returned when specific food groups were reintroduced.[9]

Allergic esophagitis has not been reported to be a causative factor in extra-esophageal disease; however, it is reasonable to assume that just as GERD plays a role in certain laryngeal disorders, so could allergic (or eosinophilic) esophagitis. A recent report documented the association of laryngeal clefts and eosinophilic gastroenteritis.[10] The following case report describes a child with laryngeal inflammation initially thought to be consistent with GERD who did not respond to aggressive antireflux therapy, but rather, responded to therapy used to treat allergy.

CASE REPORT

A 2-year-old girl who had been born prematurely at 32 weeks required intubation for 3 weeks. Upon extubation, she had mild to moderate stridor that was exacerbated by upper respiratory tract infections. She also developed intolerance to many food products, suffering from choking and vomiting episodes. She received a diagnosis of SGS and underwent multiple laser procedures at an outside institution. Upon arrival at Children's Hospital Medical Center in Cincinnati, she underwent endoscopy that revealed a Myer-Cotton grade III SGS (see Figure, A). Her hy-

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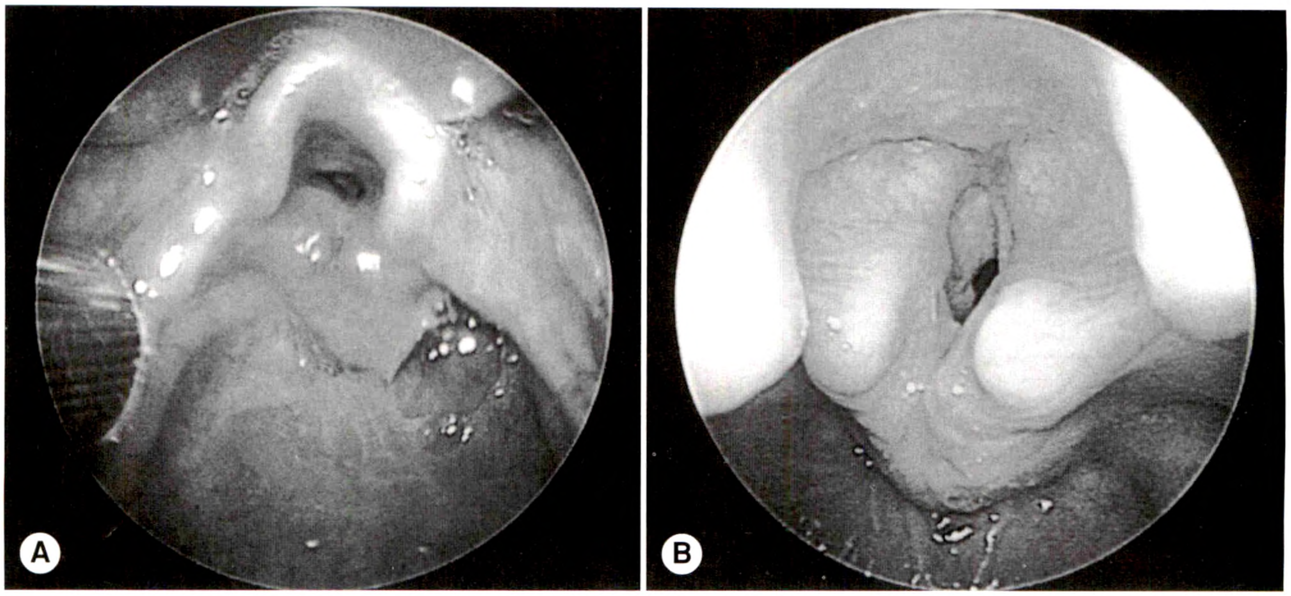

Endoscopic views of larynx of patient **A**) before and **B**) after treatment for allergic esophagitis.

popharyngeal and postcricoid regions showed inflammation that suggested GERD. Flexible esophagoscopy and biopsies of the esophagus were performed. A double-probe pH monitor was placed with the upper probe positioned in the base of the pyriform fossa and the lower probe at the level of the third thoracic vertebra. The patient then underwent a functional endoscopic evaluation of swallowing that revealed no signs of penetration or aspiration and revealed an essentially normal pharyngeal swallow. The pH probe monitoring was performed overnight for a total recording time of 15 hours and revealed no episodes of a pH of <4 in the upper esophagus, and a pH of <4 in the lower esophagus for only 1% of the recording time. The biopsy results were significant for the finding of eosinophilia (10 to 12 eosinophils per high-power field [HPF]).

Given the clinical appearance of the child's postcricoid region and hypopharynx, the decision was made to start her on an empiric course of Cisapride (0.3 mg/kg per day divided into 4 daily administrations) and Prilosec (1 mg/kg per day in 4 daily administrations) and then to proceed with surgical correction of her airway. The patient underwent a single-stage cricotracheal resection and was intubated for 6 days. After the operation, she was maintained on her anti-reflux medications; over the ensuing weeks, the SGS recurred and did not respond to serial dilations. Her postoperative course was complicated by difficulty with oral intake, as she could not tolerate many different food products without retching and vomiting spells. She underwent allergy testing, which revealed an essentially normal complete blood count other than an elevated IgE level of 38 IU/mL. (In our laboratory, the mean normative value for the serum IgE level in a 2-year-old is 3 IU/mL, and >2 SD from the mean, or >29.5 IU/mL, represents an elevated laboratory value.) Skin testing showed evidence of allergy. Radioallergosorbent testing showed a positive allergic response to peanuts, milk, and walnuts.

The patient then underwent a single-stage laryngotracheal reconstruction with costal cartilage grafting. A pH probe was placed during the operation and again failed to demonstrate any significant GERD over the next 48 hours. The patient's postoperative course was stormy, as she quickly redeveloped tightness of breathing and intermittent stridor. She suffered an anaphylactic reaction to a peanut that required emergent intubation and medical therapy.

In light of the strong history of allergic responses, the patient was placed on 0.5 mg/kg Decadron given every other day for 1 month and then tapered off. Over this period, her symptoms markedly improved; her ability to eat food without event improved, as did her airway symptoms. Repeat endoscopy showed a significantly improved postcricoid region with minimal inflammation or edema (see Figure, B). Biopsy of the esophageal mucosa revealed an absence of eosinophilia. She persisted with a grade II SGS. Over the 6-week course of therapy, she had developed side effects to the steroid treatment, including significant weight gain with water retention. At the 6-week follow-up after steroid discontinuation, the side effects of the steroids had significantly diminished, but so had their effect. Again, she was experiencing tightness of breathing and retching and vomiting with feeding.

The patient has been placed on an aerosolized steroid regimen and a strict diet following the recommendations of Kelly et al.[9] Her symptoms markedly improved over a 2-week period. She currently is undergoing further allergy testing and dietary evaluation. The decision has been made to delay further surgery pending medical management of her allergic symptoms.

DISCUSSION

There are many anecdotal reports, as well as published cases, of children with signs and symptoms of GERD who fail to respond to either maximum medical therapy or surgical therapy.[6,9] A subset of these patients have esophageal biopsies that document eosinophilia. There has been divergence in the literature as to how to interpret this finding. Winter et al[1] noted that eosinophilia was an early sign of GERD, and that the more prominent the eosinophilia, the more severe the GERD. Their patients, however, generally had fewer than 5 eosinophils per HPF. Other studies have used esophageal eosinophilia as a marker for GERD.[11-13]

Recently, more severe eosinophilia has been recognized to be due to allergy and other nonreflux causes. Liacouras et al[5,6] examined a cohort of children with esophageal eosinophilia and GERD symptoms who did not respond to medical therapy or Nissen fundoplication. They found that children with "allergic esophagitis" who responded to corticosteroid treatment had a greater eosinophilia than did children with esophageal eosinophilia and GERD who responded to traditional medical treatment.[5] Other groups have described similar cohorts of patients[8]; Ruchelli et al[7] used the number of eosinophils per HPF (ie, >7) to predict the success of antireflux therapy, and posited that alternative medical therapies aimed at allergic causes might well be effective.

The role of GERD in the genesis and outcome of upper airway disease processes has been well documented, but remains controversial. Laryngeal manifestations of GERD have been postulated since Cherry and Margulies[14] described a possible relationship between GERD and the development of laryngeal contact ulcers. Since that time, GERD has been associated with a host of respiratory manifestations.[15,16] There have been several anecdotal reports of the success of preoperative medical treatment of GERD with respect to the outcome of surgical repair of laryngotracheal stenosis.[17-19] These reports contrast with the 1996 study by Zalzal et al,[20] which denies an association between GERD and the outcome of laryngotracheal stenosis surgery.

Our case report portrays a child with acquired SGS and marked symptoms of GERD. The complicating factors were the failure of double-probe pH studies to measure acid reflux, as well as the inability of fairly aggressive medical antireflux therapy to control the symptoms. Surgical treatment of GERD was considered, but in the face of the negative pH probe tests, it was difficult to justify. After formal allergy testing and a review of esophageal biopsies that showed 10 to 12 eosinophils per HPF, a trial of corticosteroid therapy was implemented with marked improvement of symptoms. (We are using the definition of Ruchelli et al[7] of >7 eosinophils per HPF as a marker for allergic esophagitis.) Repeat esophageal biopsies showed an absence of eosinophilia. Symptoms (both airway and gastrointestinal) returned upon withdrawal of the steroid regimen, and as the patient developed minor side effects after a 1-month course of steroids, a longer treatment regimen with this course of therapy seemed unreasonable. Aerosolized corticosteroid therapy presents an untested but potential alternate treatment and has been offered as an option to the parents of children with allergic esophagitis.[21]

Another treatment option, which has been suggested by the work of Kelly et al,[9] is dietary therapy. Kelly et al proposed the hypothesis that symptoms of allergic esophagitis were a response to the ingestion of intact dietary proteins. When they tested this hypothesis by removing all intact dietary proteins from 10 children's diets for a period of time and giving them Neocate or Neocate-1 Plus supplement, the childrens' symptoms improved. This treatment option needs further evaluation, but holds promise.

CONCLUSIONS

There is a cohort of patients with signs and symptoms of allergic esophagitis who respond to corticosteroid and/or steroid therapy. We present the case of a child with acquired SGS and signs and symptoms of allergic esophagitis. This represents the first association between allergic esophagitis and a laryngeal disorder such as SGS. For children with extraesophageal disease suspected to be related to or aggravated by GERD, in which traditional medical and/or surgical therapy fails to relieve the symptoms, a diagnosis of allergic esophagitis should be considered.

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EXPRESSION OF GLUCOCORTICOID RECEPTORS AND CYCLOOXYGENASE-2 IN NASAL POLYPS FROM NONALLERGIC PATIENTS

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Glucocorticoid treatment has been widely used to suppress inflammatory and immune responses. However, from a clinical point of view, its efficacy in the treatment of nasal polyposis seems to vary individually from patient to patient. In the present study, we examined the presence of glucocorticoid receptors (GRs) and cyclooxygenase-2 (COX-2) in the nasal polyps of nonallergic patients as compared with normal controls. Reverse transcription–polymerase chain reaction analyses revealed the presence of both GR messenger RNA and COX-2 messenger RNA expression in nasal polyps from nonallergic patients, as well as in normal nasal mucosa from controls. Consistent with this finding, immunohistochemical analysis demonstrated that GRs and COX-2 were labeled in both tissues. In nasal polyps, GR labeling was associated with the cytoplasm and nucleus of surface mucosa, submucosal glands, endothelial cells, and inflammatory cells. Importantly, COX-2 labeling was seen in the cytoplasm of surface mucosa, submucosal glands, and the vascular wall in nasal polyps. In contrast, in normal nasal tissues, COX-2 labeling was only found in the vascular wall, and the expression was weaker — a finding demonstrating that COX-2 is upregulated in nasal polyps. Therefore, 1) the presence of GRs and COX-2 in nasal polyps from nonallergic patients, as well as upregulation of COX-2 expression, suggests that inflammation may play an important role in the pathophysiology of nasal polyps; and 2) glucocorticoid could be a potential treatment method for suppressing inflammatory processes.

KEY WORDS — cyclooxygenase-2, glucocorticoid receptor, nasal polyp, nonallergic rhinitis.

INTRODUCTION

Nonallergic rhinosinusitis is a chronic disease of unknown cause that is characterized by inflammatory cells in the mucous membranes of the nose and paranasal sinuses and by frequent occurrence of nasal polyps. The pathogenesis of nasal polyps is not well understood, but some evidence demonstrates that inflammation may play a role.[1-3] Glucocorticoid treatment has been widely used in relieving the symptoms and signs of nasal polyposis; however, from a clinical point of view, its efficacy has not been proven and seems to be quite variable.

Glucocorticoids have a multifactorial effect on various aspects of the inflammatory reaction in the airway mucosa, initiated by their binding to specific cytoplasmic glucocorticoid receptors (GRs).[4] At the cellular level, most known effects of glucocorticoids are mediated by an approximately 94 kd intracellular protein in the GRs.[5] The drug-receptor complex modifies gene transcription and induces a change of protein synthesis in the cell that is responsible for the clinical effect that becomes manifest in treating nasal polyps.[5]

Cyclooxygenase (COX) is a key enzyme required for prostaglandin (PG) synthesis. An arachidonic acid metabolite, PG is one of the important mediators in inflammatory processes produced by the COX pathway.[6] It has been shown that the expression of COX-2, an inducible COX isoform, is upregulated in inflammatory reactions, whereas its expression is reduced with glucocorticoid treatment.[7,8]

Thus, in the present study, we examined nasal polyps from nonallergic patients in order to test whether GRs or COX-2 are expressed in nasal polyps. We wanted to elucidate 1) whether inflammation mediated by the production of PG by COX-2 is involved in the pathophysiology of nasal polyps from nonallergic patients; and 2) whether glucocorticoid treatment is a reasonable means to suppress inflammation in nasal polyps in nonallergic patients. Moreover, we examined whether GRs or COX-2 are expressed in normal nasal mucosa. We used reverse transcription–polymerase chain reaction (RT-PCR) analyses and immunohistochemical analysis.

MATERIAL AND METHODS

Patient Selection. Patients with nonallergic nasal polyposis (n = 20) were selected on the basis of a

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CHARACTERISTICS OF PATIENTS WHO UNDERWENT POLYPECTOMY AND FUNCTIONAL ENDOSCOPIC SINUS SURGERY

Patient No.	*Age (y)*	*Sex*	*Diseased Sinuses*
1	58	M	M, E, S
2	76	F	M, E, S
3	62	M	M, E
4	40	M	M, E
5	53	F	M
6	46	M	M, E, S
7	54	M	M, E
8	25	F	M, E
9	13	M	M, E, S
10	14	M	M, E
11	16	F	M, E
12	25	F	M, E
13	50	M	M, E
14	43	F	M, E
15	31	F	M
16	18	M	M, E, S
17	29	M	M
18	27	F	M, E
19	13	F	M, E, S
20	41	M	M, E, S

M — maxillary, E — ethmoid, S — sphenoid.

history of more than 1 year of chronic paranasal sinusitis and nasal polyps with 1 or more of the following symptoms: chronic perennial nasal congestion, chronic rhinorrhea, chronic posterior nasal drip, anosmia, and pain or discomfort in the regions of the sinuses. All patients had nasal polyps and radiographic evidence of sinusitis on a recently taken sinus computed tomographic scan (see Table). Allergic and aspirin-intolerant patients were excluded. The diagnosis of allergy was made on the basis of both a history of allergy and the results of the skin test. Patients were also excluded if they had received systemic or topical steroids within a month. The diagnosis of aspirin intolerance was made on the basis of both a history of acute wheezing, rhinorrhea, urticaria, angioedema, or symptoms of shock precipitated by nonsteroidal anti-inflammatory drugs and a positive aspirin provocation test. Each patient signed an informed consent that was approved by the ethics committee of our hospital. Normal (control) nasal mucosa was obtained from the inferior turbinate of persons subjected to septorhinoplasty (n = 4). These controls had no allergy history, allergic symptoms, or nasal polyps.

Nasal Polypectomy and Tissue Handling. Nasal polypectomy was performed before functional endoscopic sinus surgery under general anesthesia. Before surgery, the nasal cavity was packed with a pledget soaked with 2% lidocaine mixed in a 1:100,000 solution. The polyps were removed with a sickle knife and cutting forceps and were immediately submerged in RNAlater (Ambion, Inc, Austin, Texas) for storage without jeopardizing the quality or quantity of RNA.

RNA Extraction. The nasal polyp specimens were homogenized with a tissue mincer in a solution containing 4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, pH 7, 0.5% sarcosyl, 0.1% β-mercaptoethanol. For the extraction of RNA, the procedure described by Chomczynski and Sacchi[9] was modified as follows. After homogenization of the tissues, the homogenate was incubated on ice for 10 minutes. Sequentially, one 10th volume of 2 mol/L sodium acetate, pH 4, 1 volume of water-soluble phenol, and one 5th volume of a chloroform-isoamylalcohol mixture (49:1) were added to the homogenate, with thorough mixing by inversion after the addition of each reagent. The final suspension was shaken vigorously for 30 seconds and cooled on ice for at least 15 minutes. The samples were centrifuged at 12,000 rpm for 15 minutes at 4°C. The aqueous phase was then transferred to a fresh tube, mixed with 1 volume of isopropanol, and kept at −20°C for at least 1 hour to precipitate RNA. After centrifugation at 12,000 rpm for 15 minutes at 4°C, the RNA pellet was washed once by vortexing in 70% ethanol for 30 seconds, sedimented, air-dried for 5 minutes, dissolved in 30 to 50 μL of diethyl pyrocarbonate–treated water, and stored at −70°C. The quantity and quality of the isolated RNA were determined by absorbance at 260 nm and 280 nm.

Reverse Transcription–Polymerase Chain Reaction. Reverse transcription–polymerase chain reaction was performed with the Titan One Tube RT-PCR kit from Roche (Roche, Mannheim, Germany). One microgram of total RNA was used as template in the RT-PCR. The 50-μL total RT-PCR volume consisted of 10 μL 5× RT-PCR buffer, 2.5 mmol/L magnesium chloride, 5 units RNase inhibitor, 5 mmol/L dithiothreitol, 0.3 mmol/L each deoxynucleotide triphosphate, 20 pmol of upstream and downstream primers, and 1 μL enzyme mixture (AMV reverse transcriptase and Taq/Pwo enzyme blend). The RT reaction and the PCR amplification were performed sequentially in a single tube without interruption in a Perkin Elmer 2400 PCR thermocycler under the following thermocycling conditions: 50°C for 30 minutes (RT); 94°C for 2 minutes (initial denaturation); 94°C for 30 seconds, 55°C to 60°C for 30 seconds, 68°C for 45 seconds (10 cycles); 94°C for 30 seconds, 55°C to 60°C for 30 seconds, 68°C for 45 seconds (cycle elongation of 5 seconds for each cycle; 25 cycles); 68°C for 7 minutes (final extension).

The RT-PCR amplification of GRs was carried out

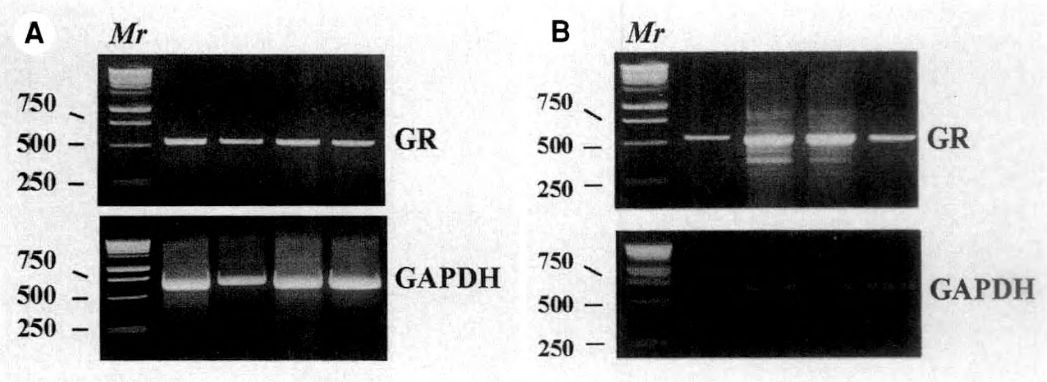

Fig 1. Expression of glucocorticoid receptor (GR) messenger RNA (mRNA) in nasal polyps and normal nasal mucosa from controls. Total RNA was extracted from both nasal polyps from patients with nonallergic rhinitis and normal nasal mucosa and subjected to reverse transcription–polymerase chain reaction (RT-PCR). Representative photographs of PCR products of **A**) nasal polyps and **B**) normal control mucosa are shown. Mr — molecular weight; GAPDH — glyceraldehyde phosphate dehydrogenase.

at an annealing temperature of 55°C. The primer sequences were as follows: GR (sense) 5'-TGCAGCAGTGAAATGGGCAA-3' (position 1848-1867); GR (antisense) 5'-GGGAATTCAATACTCATGGTC-3' (position 2361-2381); the predicted size of the product was 534 bp. The RT-PCR amplification of COX-2 was carried out at an annealing temperature of 60°C. The primer sequences were as follows: COX-2 (sense) 5'-TTCAAATGAGATTGTGGGAAAATTGCT-3'; COX-2 (antisense) 5'- AGATCATCTCTGCCTGAGTATCTT-3'; the predicted size of the product was 301 bp. The integrity of the RNA obtained from the clinical samples was confirmed by determining the presence of glyceraldehyde phosphate dehydrogenase (GAPDH) messenger RNA (mRNA) in the same samples with primers GAPDH (sense) 5'-CCACCCATGGCAAATTCCATGGCA-3' and GAPDH (antisense) 5'-TCTAGACGGCAGGTCAGGTCCACC-3'. The predicted size of the fragment was 593 bp. The amplified products were subjected to electrophoresis on 2% agarose gels containing ethidium bromide.

Histopathologic Analysis. Biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Five-micron-thick sections were made from each paraffin block. The sections were initially stained with hematoxylin and eosin for routine histologic analysis.

Immunohistochemical Analysis. Tissue sections of each specimen were mounted on positively charged slides, deparaffinized in xylene, and rehydrated through decreasing concentrations of ethanol. The slides were immersed in a pressure cooker with 0.01 mol/L citrate buffer (pH 6.0) in the autoclave for 3 to 5 minutes, and then blocked with normal horse serum and incubated for 1 hour at room temperature with rabbit polyclonal anti-mouse GRs against both GR-α and GR-β (Santa Cruz Biotechnology, Santa Cruz, California) at a 1:100 dilution and rabbit polyclonal anti-human COX-2 (Cayman Chemical Company, Ann Arbor, Michigan) at a 1:500 dilution. Staining was achieved with a Dako LSAB+ kit (Dako, Santa Barbara, California) and developed with 3-amino-9-ethylcarbazole. The sections were counterstained for 5 minutes with Mayer's hematoxylin and mounted.

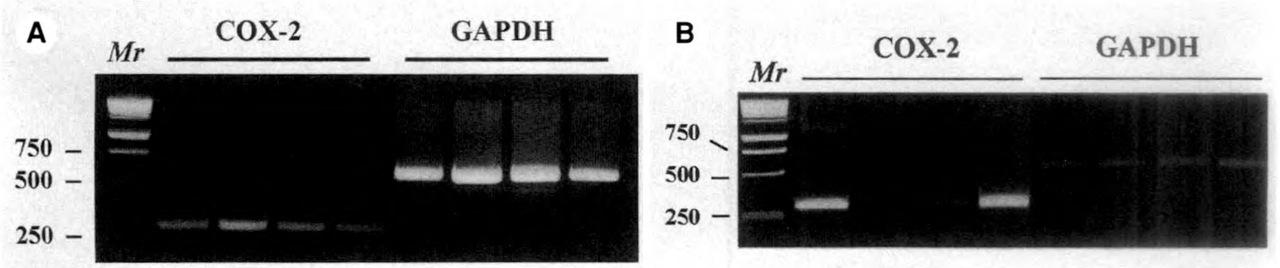

Fig 2. Expression of cyclooxygenase-2 (COX-2) mRNA in nasal polyps and in normal nasal mucosa. Total RNA was extracted from both nasal polyps from patients with nonallergic rhinitis and normal controls and subjected to RT-PCR. Representative photographs of PCR products of **A**) nasal polyps and **B**) normal control mucosa are shown. Samples in lanes 5 to 8 correspond to those in lanes 1 to 4, respectively.

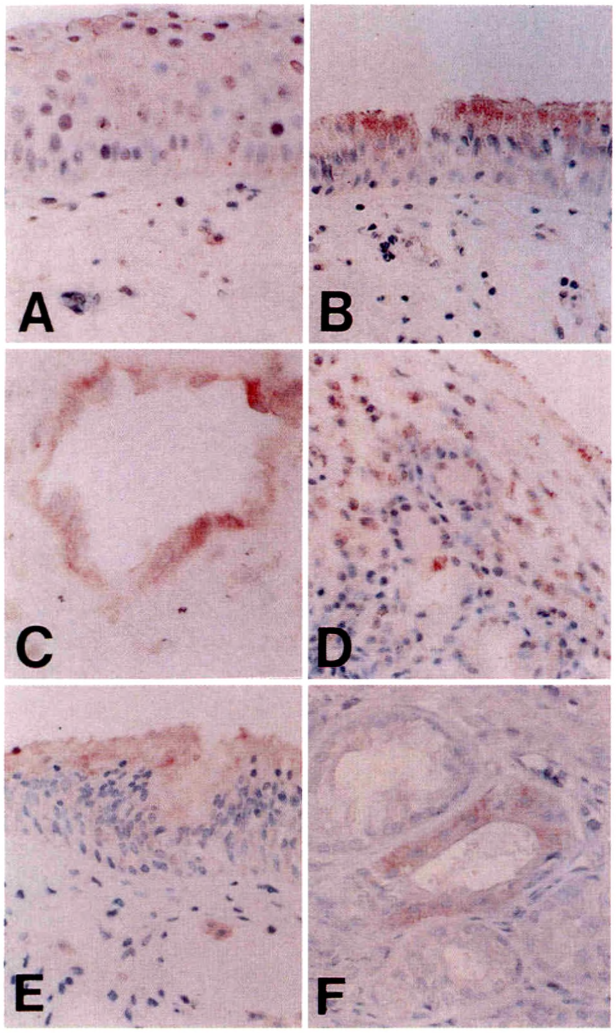

Fig 3. Immunohistochemical sections of GRs in nasal polyps from patients and normal nasal mucosa from controls (original ×200, except for C, in which original ×400). Nasal polyps are associated with expression of GRs in **A**) squamous metaplasia, **B**) surface mucosa, **C**) endothelial cells, and **D**) inflammatory cells of respiratory epithelium. Normal nasal tissues show immunoreactivity in **E**) respiratory epithelium and **F**) submucosal glands.

RESULTS

Expression of GRs. To examine the expression of GRs in nasal polyps from nonallergic patients and in normal nasal mucosa from normal controls, we performed RT-PCR using GR primers. The RT-PCR demonstrated that the 534 bp PCR product for GR mRNA was observed in both nasal polyps and normal nasal mucosa (Fig 1). This finding suggested that GR mRNA is expressed in both nonallergic patients and normal controls, and was confirmed by immunohistochemical analysis (see below).

Expression of COX-2. The expression of COX-2 mRNA was examined by RT-PCR analyses both in nasal polyps from nonallergic patients and in normal nasal mucosa from controls. The 301 bp PCR product for COX-2 mRNA was detected in nasal polyps and normal nasal mucosa (Fig 2). However, the immunolabeling of COX-2 expression was much weaker in the normal nasal mucosa from controls than in nasal polyps (see below), suggesting low abundance.

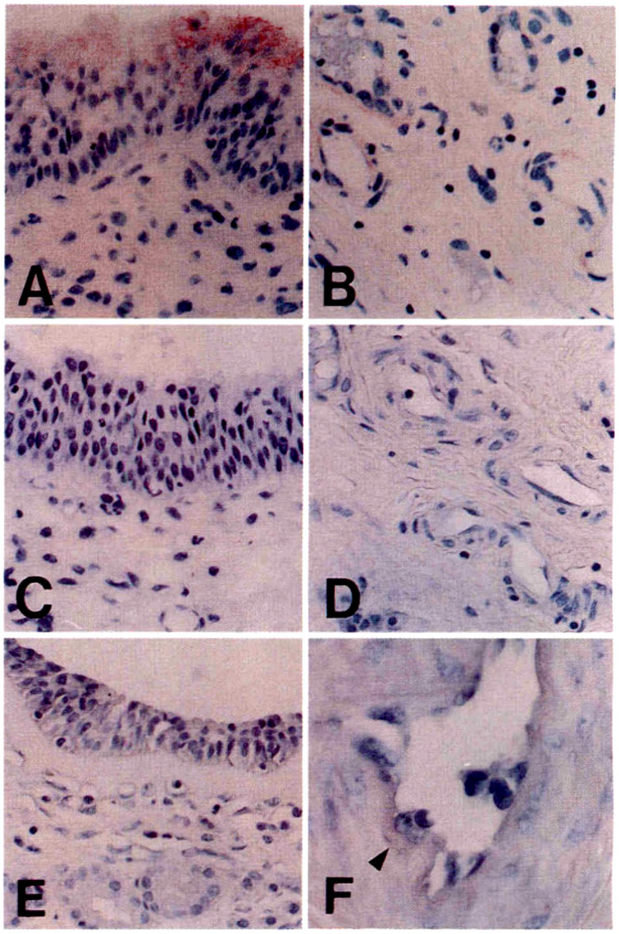

Fig 4. Immunohistochemical sections of COX-2 in nasal polyps and normal nasal mucosa from controls (original ×200, except for F, in which original ×400). Nasal polyps are associated with expression of COX-2 in **A**) surface mucosa and **B**) vascular smooth muscle. **C,D**) Immunoreactivity was absent in negative control using rabbit IgG in place of primary antibody. **E,F**) Normal nasal tissues show immunoreactivity only in vascular wall (arrowhead).

Immunohistochemical Analysis of GRs and COX-2. In nasal polyps from nonallergic patients, GR labeling was observed in all examined tissue specimens. The labeling was localized in the cytoplasm and nucleus of the surface mucosa, submucosal glands, endothelial cells, and inflammatory cells (Fig 3). Particularly, the staining intensity of the GRs in the surface mucosa was stronger than that of other areas. Consistent with this finding, GRs were similarly expressed in normal nasal mucosa (Fig 3).

The COX-2 labeling in nasal polyps was localized focally in the cytoplasm of the surface mucosa, submucosal glands, vascular wall, and mast cells with similar staining intensity (Fig 4). In contrast, in normal nasal mucosa, COX-2 was weakly labeled only in the vascular wall (Fig 4), and no labeling was observed in the other parts of the nasal tissues in which COX-2 labeling was evident in the nasal polyps (Fig 4).

DISCUSSION

Nasal polyps commonly occur with various upper airway diseases like allergic rhinitis, nonallergic rhinitis, and asthma. Several pathogenic factors, such as inflammation, allergy, vasomotor disturbances, and mechanical factors, have been suggested. Among them, inflammation has widely been investigated as a possible factor in the pathogenesis of nasal polyps. To elucidate that inflammation is one of the possible factors in the pathogenesis of nasal polyps, the production of a number of cytokines in nasal polyps has been demonstrated, and their possible roles in the pathogenesis of polyposis have been investigated.[10] These studies have suggested the hypothesis that nasal polyposis may be associated with a self-perpetuating inflammatory reaction caused by overproduction of cytokines and tissue-derived growth factors in an inductive environment.[10]

Among these cytokines, PG, which is an arachidonic acid metabolite, is one of the important mediators in inflammatory processes produced by the COX pathway. Thus, glucocorticoid has widely been used to suppress inflammation, as it may inhibit the synthesis of cytokines not only from lymphocytes, but also from alternative sources such as epithelial cells.[11] However, the efficacy of glucocorticoid treatment is still controversial. In the present study, because of the inconsistent effect of glucocorticoid on the nasal polyps from patients with nonallergic rhinitis, we examined the presence and localization of GRs and of COX-2 in nasal polyps by using RT-PCR and immunohistochemical analyses. Moreover, the expression of GRs and COX-2 was compared with that in normal nasal mucosa from controls to investigate their possible roles in the pathogenesis of nasal polyposis. This study may elucidate whether inflammation is mainly involved in the pathophysiology of nasal polyps.

Glucocorticoid receptors, which belong to the phylogenetically conserved superfamily of nuclear hormone receptors (ie, for mineralocorticoid, androgen, progestin, estrogen, and thyroid hormone), mediate most of the known effects of glucocorticoids.[5] The mechanism of steroid action is known to be mediated through the steroid-receptor complex; namely, glucocorticoid action is initiated by its binding to a specific cytoplasmic GR and is followed by a conformational change in glucocorticoid, and the hormone-receptor complex translocates to the nucleus and binds to its acceptor sites in DNA.[12,13]

Kai et al[14] demonstrated that glucocorticoid affinity sites in normal nasal tissue are present in epithelium, veins, leukocytes, and glands by using radiolabeled synthetic glucocorticoid. We demonstrated that GR mRNA is expressed in both nasal polyps and normal nasal mucosa by RT-PCR analyses. Moreover, the cellular and subcellular localization of GRs observed by immunohistochemical analysis was found to be in the cytoplasm and nucleus of the surface epithelium, submucosal glands, endothelial cells, and inflammatory cells in both tissues. This finding was consistent with those of previous studies and suggests that GRs are present in normal tissues, as well as in nasal polyps. The expression of GRs in submucosal glands therefore suggests that glucocorticoid treatment may reduce the sensory response to stimulation of the glands, possibly through the reduction of the receptor sensitivity of the sensory nerve.[15] Moreover, the presence of GRs in the endothelium may result in the reduction of endothelial permeability,[12,16] and the existence of GRs in the cytoplasm and nucleus of surface epithelium may imply that glucocorticoid action in the epithelium is mediated directly, as well as indirectly, through the steroid-receptor complex.

It is generally understood that steroid reduces the spontaneous release of COX metabolites, including PGs. Prostaglandins are important mediators for inflammation whose synthesis is initiated by the release of arachidonic acid from cell membranes.[6] Cyclooxygenase, a key enzyme required for PG synthesis, is transcribed from 2 distinct genes, COX-1 and COX-2. The first is expressed constitutively in most tissues, whereas COX-2, which was initially identified as an immediate or early growth response gene,[17-19] is mainly induced by a variety of stimuli. Cyclooxygenase has widely been analyzed with regard to its physiological or pathophysiological relevance to human disease models.

For example, the presence of COX expression has previously been demonstrated in the airway mucosa, as well as in cultured epithelial cells from human lower airways and pulmonary fibroblasts.[20-23] Particularly, Demoly et al[21] demonstrated that COX-1 and COX-2 are expressed in normal nasal mucosa, as well as in mucosae from patients with pathophysiological conditions (eg, chronic sinusitis, and nasal polyps from allergic and nonallergic patients). However, they found that expression of COX-1 and COX-2 in these diseases was not upregulated as compared to normal controls.[21] In this study, we observed the expression of COX-2 mRNA in nasal polyps from patients with nonallergic rhinitis, as well as in nomal mucosa, by RT-PCR analyses. We, however, demonstrated that COX-2 expression in normal nasal mucosa was much weaker and that labeling was only seen in the vascular wall, with no labeling observed in other parts of the nasal tissues, whereas COX-2 labeling was evident in nasal polyps from nonallergic patients (Fig 4). This finding may indicate that COX-2 expression is upregulated in nasal polyps as compared to normal nasal mucosa and may suggest an important role in the pathogenesis of nasal polyps.

The pathophysiological significance of COX-2 expression in nasal polyps is poorly understood, although such PGs as PGD2, PGF2α, and PGE2 were found to be produced in nasal polyps.[3] Hence, these PGs are possibly involved in the modulation of mucosal inflammation associated with chronic sinusitis, which is commonly accompanied with polyps. Consistent with this hypothesis, Crofford et al[24] showed that COX-2 expression was dramatically suppressed by dexamethasone in rheumatoid synovial explant tissues and cultured synoviocytes. As steroids downregulate many genes involved in inflammatory responses, the significant inhibition of COX-2 mRNA appears likely to play a significant role in the suppression of inflammation.[24] As we demonstrated that COX-2 expression in nasal polyps from nonallergic rhinosinusitis was increased compared to that of controls, it can be speculated that expression of COX-2 in nasal polyps presumably is involved in their pathogenesis. Therefore, further studies are needed to directly examine the effectiveness of corticosteroid treatment for nasal polyposis.

It should be emphasized that aspirin-sensitive patients with nasal polyposis were excluded from this study because aspirin may have an influence on the expression of COX-2, as discussed below. Sousa et al,[7] using immunohistochemical analysis, observed that COX-1 and COX-2 are present in biopsy specimens from human bronchi. They found a significant increase in the number of cells expressing COX-2 in asthmatic subjects as compared to healthy individuals. Moreover, COX-2 expression was increased in aspirin-sensitive patients as compared to aspirin-tolerant patients.[7] In contrast, Picado et al[2] observed that the expression of COX-2 was downregulated in nasal polyps from aspirin-sensitive asthmatics. Another study revealed that COX-1 and COX-2 expression in bronchial tissues showed no difference in asthma patients versus normal controls.[22]

In summary, we demonstrated the presence of both GR mRNA and COX-2 mRNA in nasal polyps from nonallergic patients, as well as in normal nasal mucosa, by RT-PCR analyses. Similarly, immunohistochemical analysis of sections from the nasal polyps revealed the presence of GRs and COX-2. In normal nasal mucosa, GR expression was similar to that in polyps, whereas COX-2 expression was observed only in the vascular wall, albeit at very low levels, on immunohistochemical analysis, and other parts exhibited no labeling. The presence of GRs and COX-2 in nasal polyps from nonallergic patients, as well as upregulation of COX-2 expression, suggests that inflammation may play a role in the pathophysiology of nasal polyps. Hence, glucocorticoid treatment could be a potential treatment for suppressing inflammatory processes.

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AUDIOLOGICAL ASSESSMENT IN RAMSAY HUNT SYNDROME

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Ramsay Hunt syndrome is known to cause symptoms and signs of vestibulocochlear dysfunction, including sensorineural hearing loss. The present study investigates the audiological features of a group of 15 patients with this syndrome. A complete otolaryngological, neurologic, and audiological workup was performed in every patient, including auditory brain stem response measurements and recording of transiently evoked otoacoustic emissions. In most patients, some degree of hearing loss was evident, and abnormal latencies and interpeak latencies of the auditory brain stem response, or even absence of the waves, were observed. Transiently evoked otoacoustic emissions were present in only 6 cases, and caloric tests showed unilateral weakness in the majority of the patients. In all of the performed tests, abnormalities were present only on the affected side. The audiological data suggested cochlear or retrocochlear involvement or involvement at more than one site along the auditory pathway.

KEY WORDS — auditory brain stem response, cochlear lesion, hearing loss, herpes zoster oticus, retrocochlear lesion, transiently evoked otoacoustic emission.

INTRODUCTION

Varicella-zoster virus (VZV) is a member of the family of human herpesviruses and is the etiologic agent of varicella (chickenpox) and herpes zoster (shingles).[1,2] This virus is a ubiquitous infection agent, and it is known that at least 95% of the adult population is infected with it, as evidenced both by circulating virus-specific antibodies and the known lifetime carriage of the virus in a latent state. Varicella results from initial exposure to VZV. Herpes zoster is the clinical expression of the focal reactivation of VZV in dorsal root ganglia, which have remained dormant from the time of varicella infection. However, occasional cases are attributed to reinfection of an immunized host by further contact with a case of varicella or zoster.[3] Epidemiological data suggest that zoster is an infection whose likelihood increases directly in proportion to the age of the individual and inversely with the individual immune competence.

Herpes zoster is characterized by pain and a vesicular eruption on an erythematous base in one or several dermatomes. The trigeminal ganglia are the most frequently involved in herpes. The head is involved in approximately 19% of cases, and nearly all of these are in the trigeminal distribution.[4] Neurologic complications of herpes zoster infection are common,[5] including cerebrospinal fluid changes, post-herpetic neuralgia, peripheral motor neuropathy, various cranial nerve palsies, myelitis, encephalitis, thrombotic cerebral vasculopathy, acute ascending polyradiculitis, and aseptic meningitis. In 1907, Ramsay Hunt[6] was the first to hypothesize that VZV infection in the geniculate ganglion was responsible for the clinical syndrome consisting of herpetic eruptions on the auricle, facial paralysis, and vestibulocochlear dysfunction, and introduced the term herpes zoster oticus. However, it was later recognized that the true extent of the disease was greater, and it was suggested[7] that the disease should be labeled herpes zoster cephalicus. Nevertheless, the term Ramsay Hunt syndrome is widely used today.

Patients with herpes zoster oticus present with ear pain, facial paralysis, dysgeusia, tinnitus, hearing loss, hyperacusis, and vertigo.[8] Auditory tests usually suggest either cochlear or retrocochlear involvement, but involvement in both sites is also possible. These findings are consistent with the pathological findings at autopsy and with findings on magnetic resonance imaging[9] that suggested a variety of lesions along the auditory pathway. Vestibular involvement may be severe, and balance problems in the elderly are often incapacitating.[10] Adjacent cranial nerves may become involved during the inflammation process, and pharyngeal vesicles and accompanying pain may be evident in the distribution of the 5th, 9th, and 10th

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cranial nerves.

Although Ramsay Hunt syndrome is a clinical entity familiar to otolaryngologists, in-depth analysis of audiological findings in the disease is scarce in the existing literature.[11,12] With the development of electrophysiological methods, a more complete and objective assessment of the auditory pathway is possible. A modern diagnostic method — otoacoustic emissions — has been widely used during the past decade to study the detailed mechanical aspects of cochlear function in a noninvasive and objective manner.[13] The emissions are believed to originate from the active process of the cochlea. Outer hair cell motility produced by electromotile-related proteins generates mechanical energy within the cochlea that is re-emitted as sound from the ears by means of the middle ear system. Vibration of the tympanic membrane then produces acoustic signals — the otoacoustic emission — that can be measured with a sensitive microphone inserted into the external ear canal.

The aim of this study was to investigate the audiological profiles of a group of patients with Ramsay Hunt syndrome by using the methods of transiently evoked otoacoustic emissions (TEOAEs) and auditory brain stem response (ABR) in a complete otolaryngological, neurologic, and audiological workup.

MATERIAL AND METHODS

Fifteen patients with a clinical and biochemical diagnosis of Ramsay Hunt syndrome were studied. All patients had vesicular eruptions on the ear, facial paralysis, and symptoms of vestibulocochlear dysfunction. The patients underwent a complete otolaryngological and neurologic examination. The diagnosis of herpes zoster cephalicus was further confirmed by a fourfold or more difference between acute and convalescent complement fixation serum titers of VZV (enzyme-linked immunosorbent assay). All of the patients were hospitalized and afterward attended the outpatient clinic for at least 6 months. None of them had a history of hearing impairment or of an occupation with noise exposure, so hearing impairment from other causes could be excluded. There was no history of aminoglycoside or other ototoxic drug intake in any patient. All patients were treated with prednisone-acyclovir.[14] Prednisone was administered orally 60 mg/d in divided doses for 10 days and was decreased by 10 mg/d for the next 5 days. Acyclovir was administered orally in 800-mg doses 5 times daily for 10 days. Patients were reassessed clinically and underwent new tonic audiometric measurements within 6 months.

Audiological Investigations. Conventional pure tone audiometry was conducted in a standard soundproof booth with a 2-channel Amplaid 455 audiometer and earphones. The acoustic thresholds of each ear at frequencies of 0.25, 0.5, 1, 2, 4, and 8 kHz were measured. Measurements of acoustic immittance were performed with an Amplaid 720 clinical admittance meter, which allows the determination of acoustic reflex thresholds up to 125 dB hearing level, as well as reflex decay. Reflex activity was inferred from visual monitoring of the immittance meter. The activating stimuli were pure tones of 500, 1,000, and 2,000 Hz, presented in 5-dB steps, contralateral to the examined ear. Reflex threshold was defined as the lowest activator level that resulted in an observable meter deflection that was time-locked to stimulus presentation and reliably differentiable from any background activity.

Auditory Brain Stem Response. The ABRs were recorded by three 6-mm silver disk recording electrodes attached with and filled with adhesive conductive paste. The active electrode was placed on the mastoid process ipsilateral to click stimulation, and the reference electrode was placed high on the forehead, with the contralateral mastoid process reserved for the ground electrode. A single-channel recording was obtained with a Neuropak 2 system (Nihon Kohden, Tokyo, Japan). The stimuli were 100-μs square wave condensation clicks presented at a slow rate (10 Hz) with alternating click polarity. The standard minimum intensity of 80 dB sensation level (SL) was used when distinct waves I, III, and V were observed. In subjects with hearing loss in whom 80 dB SL did not yield a satisfactory waveform, a higher intensity (maximum of 95 dB SL) was used. The bandpass filters were set at 150 to 3,000 Hz (12 dB per octave roll-off). The analysis time was set at 10 ms, and an artifact-reference feature was set at 25 μV peak-to-peak. The derived waveforms were stored on a hard disk for further processing and off-line analysis. Two or more trials of 2,048 clicks were recorded to ensure reproducibility of the traces. The right and left ears were stimulated separately, and proper masking was applied to the ear not being tested. The reference group consisted of 20 healthy volunteers who were examined at the same laboratory under identical stimulation and recording conditions. The controls were selected from the same age group as the patients, and none of them had been receiving on a regular basis drugs that might directly influence the ABR characteristics. Good hearing was confirmed by audiometry in all members of the reference group. Single-component ABR values were considered abnormal when they were outside the average values ± 2 SD for latencies and interpeak latencies. Control and patient mean data were compared by Student's *t*-test. The following criteria were used to consider

TABLE 1. CLINICAL FEATURES AND LABORATORY FINDINGS IN PATIENTS WITH RAMSAY HUNT SYNDROME

Patient	*Age (y)*	*Sex*	*Side*	*Ear Pain*	*Hearing Loss*	*Vestibular Symptoms*	*Tinnitus*	*Cranial Polyneuropathy*	*VZV IgG Antibody (IU/mL)*	
									Acute	*Convalescent*
1	47	M	L	+	+	+	+	+ (Vsens, IXsens)	4.2	112.5
2	17	M	R	+	+	+	+	–	5.6	144.7
3	59	F	L	+	+	+	–	+ (IXsens)	32.7	214.5
4	32	M	R	+	+	–	+	–	8.0	95.4
5	64	M	R	+	+	+	+	+ (IXsens)	ND	432.8
6	24	F	L	+	+	–	+	–	15.4	212.5
7	56	F	L	+	+	+	+	+ (Vsens, IXsens)	6.0	339.8
8	39	M	L	+	+	+	–	–	7.5	95.9
9	52	F	R	+	–	+	–	+ (Vsens)	8.2	314.0
10	71	F	L	+	+	+	+	–	17.0	164.4
11	58	M	R	+	+	+	–	–	3.9	72.7
12	74	F	L	+	+	+	+	+ (IXsens)	ND	314.1
13	40	M	L	+	–	–	+	–	4.8	186.4
14	55	M	R	+	–	+	+	–	5.4	128.9
15	31	F	L	+	+	–	+	+ (IXsens)	ND	89.5

VZV — varicella-zoster virus, IgG — immunoglobulin G, sens — sensory, ND — not done.

the ABRs as abnormal: 1) absence of waves; 2) increased latencies in peaks I, III, and V; and 3) increased interpeak latencies I-III, III-V, and I-V. Amplitudes and interamplitudes were not measured because of the variability of the findings and the difficulty of obtaining definite conclusions.

Vestibular Tests. All patients underwent alternate binaural bithermal caloric irrigation with an Airmatic air caloric stimulator at 27°C and 47°C. An AC electronystagmographic recording of horizontal eye movements was made with a CNG 2-channel module computed nystagmograph (Hortmann Neurootometrie, Neckartenzlingen, Germany). Slow-phase velocity, culmination frequency, and amplitude of the caloric nystagmus were calculated with ENG Analyzer-2 software (Dr M. Enke, Hortmann AG, version 2.21). In our laboratory, a response is considered normal if it has an asymmetry of less than 20% and a directional preponderance of less than 30%.

Transiently Evoked Otoacoustic Emissions. The TEOAEs were recorded with an ILO88 Otodynamics analyzer (version 3.94 H; Otodynamics, London, England) connected to a portable personal computer, with a poststimulus analysis window of 20 ms. The testing was performed in a soundproof booth with the standard ILO adult probe with disposable tips. Meatus response monitoring was used to check the fitting conditions of the probe. The noise rejection level at the probe tip was set to 47 dB. The stimuli were half-sinusoidal clicks of 100-μs duration, presented in sequences of 4 clicks, with an interclick interval of 20 ms. The first 3 clicks had the same polarity and equal amplitude, and the fourth click was of reverse polarity and had an amplitude 3 times as large. The result was that after the responses to the 4 clicks of the sweep had been summated, the linear acoustic response was canceled and only the phase-locked, nonlinear cochlear component of the response remained. The recording bandwidth was set between 0.75 and 5 kHz, and the stimulus intensity was approximately 82 dB, in nonlinear stimulation mode. The repetition rate was 50 stimuli per second. Testing began only when a good fit was evident by the check-fit procedure and the spectrum of the stimulus waveform was as flat as possible. The sweeps of the 4 clicks were averaged and stored alternately into 2 separate buffers for comparative analysis. The stability of the stimulus, and therefore of the probe fit, was observed during the measurement as a colored light on the screen. If the light turned red, the probe was refitted and the measurement restarted. The noise rejection level was modified by the examiner during the test if it was considered necessary. The numbers of responses accepted and rejected by artifact rejection were displayed and updated during averaging. The test was concluded after 260 sweeps had been averaged from the beginning. A response spectrum of more than 3 dB (computer-determined) above the noise floor at least halfway across the test frequency bands of 1 to 2 kHz, 2 to 3 kHz, and 3 to 4 kHz was considered an emission response. Additionally, a whole reproducibility of 60% was considered necessary for the final pass. After the test, the following variables were evaluated: 1) overall response level; 2) whole reproducibility (in percent); 3) partial response levels at the frequency bands of 1 to 2, 2 to 3, 3 to 4, and 4 to 5 kHz; 4) reproducibility scores at the same frequency zones; and 5) intensity and stability of the stimulus.

TABLE 2. ACOUSTIC THRESHOLDS AND ACOUSTIC REFLEX THRESHOLDS IN DECIBELS

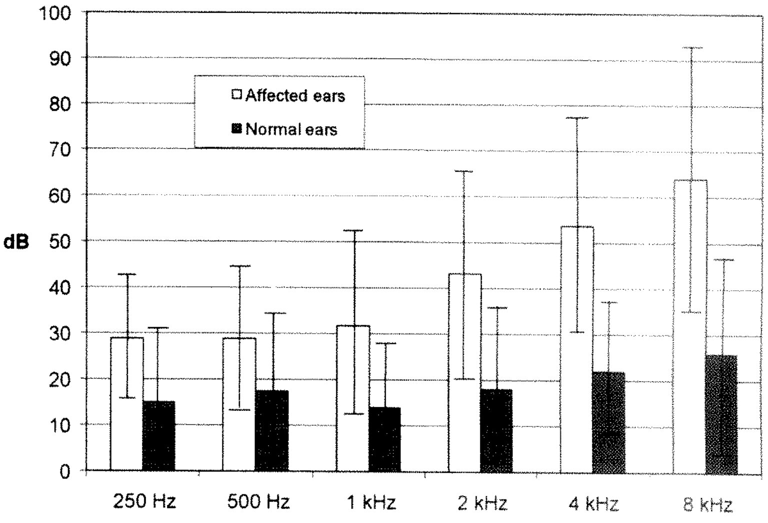
				Acoustic Thresholds						*Acoustic Reflex Thresholds*		
Patient	*Age (y)*	*Sex*	*Ear*	*0.25 kHz*	*0.5 kHz*	*1 kHz*	*2 kHz*	*4 kHz*	*8 kHz*	*0.5 kHz*	*1 kHz*	*2 kHz*
1	47	M	L	30	25	30	40	50	65	110	115	115
2	17	M	R	55	65	80	90	90	100	Abs	Abs	Abs
3	59	F	L	25	30	35	70	70	75	110	Abs	Abs
4	32	M	R	15	15	10	10	15	15	85	90	105
5	64	M	R	35	30	30	55	70	95	75	80	90
6	24	F	L	15	25	30	50	65	75	75	80	85
7	56	F	L	25	25	30	45	65	80	105	110	120
8	39	M	L	60	65	75	80	80	90	Abs	Abs	Abs
9	52	F	R	25	20	15	10	15	25	100	100	105
10	71	F	L	30	30	45	55	75	95	Abs	Abs	Abs
11	58	M	R	35	30	30	55	65	80	80	80	90
12	74	F	L	30	30	40	55	80	100	Abs	Abs	Abs
13	40	M	L	20	20	15	15	20	25	110	110	Abs
14	55	M	R	30	30	25	30	30	35	85	90	90
15	31	F	L	20	20	20	50	65	65	100	105	120

Abs — absent.

RESULTS

The main clinical features of the patients are summarized in Table 1. Eight patients were male (53%) and 7 were female (47%), ranging in age from 17 to 74 years, with a mean age of 47.9 years. All of the patients presented with partial or complete facial palsy and vesicular eruptions on the ear. The left side was involved in 9 cases and the right side in the other 6; there was no bilateral involvement. Ear pain was a common symptom in all patients, and vestibulocochlear dysfunction was evident, as subjective hearing loss, tinnitus, or vestibular symptoms were observed in most of the patients. In 1 patient (case 4), subjective hearing loss was probably a result of tinnitus, as the acoustic thresholds were within normal limits. In 7 patients, cranial polyneuritis involving the glossopharyngeal and trigeminal sensory divisions was observed. Infection with VZV was proved by a significant increase of the serum anti-VZV antibody titer during the convalescent phase of the disease as compared to the antibody titer of the acute phase.

The acoustic thresholds at 0.25, 0.5, 1, 2, 4, and 8 kHz are shown in Table 2, and the mean hearing level values at the same frequencies are represented in the Figure. In 4 patients (Nos. 4, 9, 13, and 14), the acoustic thresholds were within normal limits; the other 11 patients had various degrees of sensorineural hearing loss with no conductive element. Seven patients (Nos. 1, 3, 5, 6, 7, 11, and 15) presented with a common audiological pattern: a hearing loss on the affected side in the medium and high frequencies, either moderate or severe. In 2 more patients (Nos. 10 and 12), a similar audiological pattern was found — a hearing loss in the medium and high frequencies — but these patients were older (more than 70 years



Mean acoustic thresholds and standard deviations of affected ears (white bars) and normal ears (shaded bars) of patients with Ramsay Hunt syndrome.

TABLE 3. AUDITORY BRAIN STEM RESPONSE MEASUREMENTS

	Latencies (ms)			*Interpeak Latencies (ms)*		
Patient	*I*	*III*	*V*	*I-III*	*III-V*	*I-V*
1	1.55	**4.20**	**6.25**	**2.65**	2.05	**4.70**
2*						
3	1.67	**4.14**	**6.34**	**2.47**	**2.20**	**4.67**
4	1.82	3.75	5.55	1.93	1.80	3.73
5	1.70	3.62	5.40	1.92	1.78	3.70
6	1.75	3.47	5.64	1.72	2.02	3.89
7	**1.95**	**4.35**	**6.41**	**2.40**	2.06	**4.46**
8*						
9	1.66	**4.20**	**6.11**	**2.54**	1.91	**4.45**
10	**1.92**	**4.62**	**6.57**	**2.70**	1.95	**4.65**
11	1.67	3.64	5.61	1.97	1.97	3.94
12	*	**4.25**	**6.50**		**2.25**	
13	1.64	**4.39**	**6.12**	**2.75**	1.73	**4.48**
14	1.84	3.44	5.48	1.60	2.04	3.64
15	1.73	**4.50**	**6.43**	**2.77**	1.93	**4.70**

Abnormal values are set in bold.
*Waves absent.

of age), and a certain degree of presbycusis might have interfered. However, after we corrected for presbycusis[15] and took into consideration the hearing level of the healthy ear, an important element of pure sensorineural hearing loss remained. Finally, in another group (Nos. 2 and 8), a severe hearing loss was evident through all frequencies.

All patients' tympanograms were type A. Acoustic reflex threshold measurements were performed in all patients. In 3 patients (Nos. 5, 6, and 11), the phenomenon of recruitment was observed. In another 2 patients (Nos. 4 and 14), the acoustic reflex thresholds were normal, and in 4 more patients (Nos. 2, 8, 10, and 12), acoustic reflexes were absent. In the remaining 6 patients, the reflex thresholds were elevated or partially absent. However, no reflex decay was observed in any case. The results are also shown in Table 2.

Eleven patients had a history of vestibular symptoms, including typical vertigo, dizziness, or unsteadiness. Air caloric testing showed unilateral weakness, localized on the side of the lesion, in 9 patients, whereas the other 2 (Nos. 3 and 8) showed no response on the affected side. The symptoms improved in all patients, and when they were reexamined after 6 months, only 4 complained of occasional brief episodes of vertigo during sudden head movements.

The ABRs were studied in all patients. In 5 patients (Nos. 4, 5, 6, 11, and 14), the findings were within normal limits (Table 3). In all other patients, abnormal responses were observed, on the affected side only. In the 2 patients with severe hearing loss (Nos. 2 and 8), no ABRs could be recorded. In all other patients, wave abnormalities were observed, characterized mainly by increased latencies of waves III and V and by increased interpeak latencies, mainly the I-III and I-V intervals. Wave I was preserved in most patients, but in 2 (Nos. 7 and 10) it was delayed, and in another (No. 12) it was absent. In 2 patients with normal results of pure tone audiometry (Nos. 9 and 13), the ABRs were abnormal, despite the presence of a distinctive wave I. Three patients with medium- and high-frequency hearing loss had normal ABRs (Nos. 5, 6, and 11). Statistical processing of the values, after comparison with our normative data, showed statistically significant differences in the latencies of waves III and V and in interpeak latencies I-III and I-V (Table 4).

Transiently evoked otoacoustic emissions were absent in the 2 patients with severe hearing loss (Nos. 2 and 8). In 5 of the 8 patients with ABR abnormalities, the TEOAEs were normal, indicating adequate cochlear function; in the other 3 patients (Nos. 3, 10, and 12), they were absent. Finally, of the 5 patients with normal ABRs, only 1 (No. 4), who was already found to have normal hearing, presented with normal emissions. Absence of TEOAEs was observed in the 3 patients with hearing loss (Nos. 5, 6, and 11), but also in 1 patient with normal hearing (No. 14). The results are shown in Table 5.

After considering the whole set of audiological

TABLE 4. AUDITORY BRAIN STEM RESPONSE MEASUREMENTS IN NORMAL SUBJECTS AND IN PATIENTS WITH RAMSAY HUNT SYNDROME

		Latencies (ms)			*Interpeak Latencies (ms)*		
		I	*III*	*V*	*I-III*	*III-V*	*I-V*
Reference group (n = 20)	Mean	1.67	3.61	5.52	1.94	1.73	3.88
	SD	0.12	0.23	0.29	0.14	0.18	0.27
	Range	1.36-1.87	3.43-3.89	5.19-5.82	1.75-2.21	1.59-2.01	3.52-4.21
Ramsay Hunt syndrome (n = 15)	Mean	1.74	4.04	6.03	2.28	1.95	4.28
	SD	0.11	0.40	0.43	0.42	0.13	0.42
	Range	1.55-1.95	3.44-4.62	5.40-6.57	1.60-2.77	1.73-2.25	3.64-4.70
	p	NS	<.05	<.01	<.01	NS	<.01

NS — not significant.

TABLE 5. DATA FROM PATIENTS WITH OTOACOUSTIC EMISSIONS PRESENT

Patient	Reproducibility (%) 1-2 kHz	2-3 kHz	3-4 kHz	4-5 kHz	Whole	Response Level (dB)	Stimulus Intensity (dB)	Stimulus Stability (%)
1	59	73	66	88	71	7.8	82.4	91.9
4	91	96	99	99	93	14.4	82.3	93.0
7	45	0	89	81	67	9.7	82.2	91.7
9	72	31	94	76	72	11.2	82.3	90.4
13	97	82	84	91	87	15.2	82.4	92.0
15	83	95	98	96	85	12.3	82.2	91.4
Mean	74.5	62.8	88.3	88.5	79.1	11.7	82.3	91.7

tests performed in each patient, we may extract some results regarding the site localization of the lesion (Table 6). It appears that only 1 patient had normal findings. Five patients showed signs of retrocochlear involvement, 4 patients showed signs of cochlear involvement, and the remaining 5 patients showed signs of both.

Twelve patients were reexamined audiometrically after 6 months. In 6 of them, significant improvement or even restoration of normal hearing was noted. In another 4 (Nos. 2, 3, 8, and 10), including the 2 patients with severe hearing loss, the hearing remained impaired. Finally, 2 patients with normal initial audiograms (Nos. 9 and 13) still had normal findings. The other 2 patients with good hearing on the initial audiograms (Nos. 4 and 14) and 1 more patient with mild hearing loss (No. 1) were lost to follow-up.

DISCUSSION

Hearing loss is a common finding in patients with Ramsay Hunt syndrome. However, the incidences differ in the few series reported in the literature, from as low as 7%[16] or 19.7%[12] to as high as 85%.[11] These figures compare to our rate of 73% and refer specifically to the existence of sensorineural hearing loss and not the presence of auditory symptoms in general, which occur more often in all of the published series. It appears that these differences may be attributed to the criteria applied in order to characterize a case as Ramsay Hunt syndrome. Of course, facial palsy and vesicular eruption on the auricle are quite obvious, but the third symptom, vestibulocochlear dysfunction, is quite obscure, and some authors may include in this definition even patients with only auricular pain.[12] In our study, strict criteria for Ramsay Hunt syndrome were used, so that 2 or more definite symptoms of vestibulocochlear involvement existed and all cases were confirmed by serologic tests during the convalescent phase of the disease. Vertigo is also a common symptom and occurs in a significant number of patients with Ramsay Hunt syndrome: 72%[17] to 85%.[11] This incidence is consistent with our study, in which 11 of 15 patients (73%) had vestibular symptoms. Correlation of initial symptoms and signs of the disease with the degree of hearing

TABLE 6. SITE LOCALIZATION DETECTION BY AUDIOLOGICAL RESULTS

Patient	Hearing Loss Low Frequencies	Medium Frequencies	High Frequencies	Acoustic Reflexes	ABR	TEOAEs	Site of Lesion
1	Mild	Mild	Moderate	Elevated	Abnormal	Normal	R
2	Severe	Severe	Severe	Absent	Absent	Absent	C, R
3	Mild	Moderate	Severe	Partially absent	Abnormal	Absent	C, R
4	Normal	Normal	Normal	Normal	Normal	Normal	N
5	Mild	Moderate	Severe	Recruitment	Normal	Absent	C
6	Normal	Mild	Severe	Recruitment	Normal	Absent	C
7	Normal	Mild	Severe	Elevated	Abnormal	Normal	R
8	Severe	Severe	Severe	Absent	Absent	Absent	C, R
9	Normal	Normal	Normal	Elevated	Abnormal	Normal	R
10	Mild	Moderate	Severe	Absent	Abnormal	Absent	C, R
11	Normal	Moderate	Severe	Recruitment	Normal	Absent	C
12	Mild	Moderate	Severe	Absent	Abnormal	Absent	C, R
13	Normal	Normal	Normal	Partially absent	Abnormal	Normal	R
14	Normal	Normal	Normal	Normal	Normal	Absent	C
15	Normal	Mild	Moderate	Elevated	Abnormal	Normal	R

ABR — auditory brain stem response, TEOAEs — transiently evoked otoacoustic emissions, R — retrocochlear, C — cochlear, N — normal.

loss and the site of damage was impossible, because the number of subjects was insufficient.

The presence of cochlear and retrocochlear patterns of hearing loss has been reported by various authors. Absence of recruitment on an alternate binaural loudness balance (ABLB) test was reported in 1 patient with herpes zoster cephalicus.[18] Harbert and Young[19] reported 2 patients with Ramsay Hunt syndrome. One had poor speech discrimination, positive tone decay, and no recruitment on the short-increment sensitivity index and ABLB test, indicating a retrocochlear lesion site, which was supported further by Bekesy tracings. The second patient had hearing loss of cochlear origin, as supported by the presence of recruitment and the absence of abnormal adaptation on tone decay and Bekesy audiometry. In the more recent literature, Wayman et al,[12] in a retrospective study of 186 patients with Ramsay Hunt syndrome, of whom only 7 had been studied audiologically in detail, were able to recognize 1 patient with retrocochlear involvement, 5 with cochlear involvement, and 1 with both cochlear and retrocochlear involvement. The audiological studies used included tonic audiometry, short-increment sensitivity index, ABLB, Bekesy tracings, tone decay, speech discrimination, and ABR. Finally, Abramovich and Prasher,[11] in another detailed audiological examination of 13 patients with Ramsay Hunt syndrome, used electrocochleography and brain stem evoked potentials in addition to classic audiological tests. Seven of the 13 patients with aural herpes zoster demonstrated retrocochlear involvement, and it was concluded that hearing impairment in the disease could be classified as sensory in some cases and neural in others, with some patients showing signs of both.

To our knowledge, this study is the first to use otoacoustic emissions, in addition to ABR, in the study of patients with Ramsay Hunt syndrome, in order to estimate more accurately whether the lesion is cochlear or retrocochlear. Retrocochlear involvement was evident in 8 patients, as supported by abnormal ABR results and absent or elevated acoustic reflexes. However, in only 3 patients was hearing loss purely retrocochlear; in the other 5 patients simultaneous cochlear involvement was possible, as denoted by the absence of otoacoustic emissions. In another 3 patients, pure cochlear involvement was probable, as supported by normal ABRs, the absence of otoacoustic emissions, and the presence of recruitment. In 2 patients with severe hearing loss, otoacoustic emissions were absent and waves could not be recognized on the ABR test. In those cases, because of an additional absence of acoustic reflexes and unresponsiveness to bithermal caloric testing, a more serious infection along the auditory and vestibular pathways might have been present. Of the remaining 2 patients, both of whom presented with normal hearing as evidenced by tonic audiometry, 1 had a complete set of normal results, but the other had no otoacoustic emissions. However, this patient was a middle-aged man with acoustic thresholds that were borderline normal for his age (approximately 30 dB), so the absence of emissions is not unusual. Thus, it would not be safe to conclude cochlear damage in this patient, as these audiological results may have preceded the disease. However, the presence of other symptoms, such as tinnitus and vertigo, probably denotes vestibulocochlear involvement. It is interesting to note that other than this patient, another 3 had normal audiograms, but only 1 had normal results on all the tests, as already mentioned. The other 2 had abnormal ABR results with normal TEOAEs, indicating subclinical involvement located more centrally. It appears, therefore, that Ramsay Hunt syndrome affects the auditory system more extensively than is usually thought. Preservation of wave I and increased latencies of waves III and V could imply involvement at the level of the spiral ganglion or the eighth nerve, which might result in desynchronization and impairment of neural transmission. Brain stem involvement is also possible.

Our study lacks histopathologic data to correlate with the audiological results, but some useful conclusions may be drawn from several reports of histologic examinations of temporal bones of patients with herpes zoster oticus. Although this syndrome was called geniculate ganglionitis by Hunt himself,[6] no pathological proof of this ganglionitis was provided, and since its initial description, there has been considerable controversy over the pathological involvement of particular anatomic structures. In a classic histopathologic study,[20] inflammatory and hemorrhagic changes were found, along with necrosis of ganglia with associated neuritis and degeneration of related motor and sensory roots. Demyelination was also demonstrated in areas of mononuclear cell infiltration and microglial proliferation. However, examination of a geniculate ganglion in a case of aural herpes zoster with facial paralysis proved that it was unaffected. It was concluded that the clinical features of Ramsay Hunt syndrome could be attributed to poliomyelitis and motor neuritis, which would explain facial palsy without infection of the geniculate ganglion.[20] O'Neil,[21] who described 8 cases, argued that Hunt's assertion of geniculate ganglionitis as the pathological substrate of the syndrome was an unproven assumption. Head and Campbell[22] reported some degeneration of the gasserian ganglion of the sensory root of the trigeminal nerve and through the pons. In other histopathologic temporal bone studies, the diversity of the histopathologic findings of the disease was confirmed, and herpetic inflammatory lesions in various neural and vascular regions were reported.[7,10,23-25] The temporal bones of patients with herpes zoster oticus were examined histologically days to months after the acute illness, and involvement of both sensory and neural structures was found. In the subacute phase, the facial nerve demonstrated active neuritis with inflammatory cells throughout the facial nerve sheath and maximally near the geniculate ganglion. In addition, edema, necrosis, and hemorrhage have been found in the facial nerve. The geniculate ganglion usually shows corresponding inflammation, edema, and neuronal degeneration. In occasional cases, only part or none of the ganglion has inflammatory cells. The facial nerve nucleus in the brain stem has not been involved. In specimens examined years later, loss of facial nerve axons had occurred.

It is not yet known whether geniculate ganglionitis or facial neuritis and edema is the cause of facial palsy in Ramsay Hunt syndrome. Nevertheless, the dorsal root ganglion is known to harbor VZV during the latent state and is the tissue most severely affected during acute VZV infection. Also, on gadolinium-enhanced magnetic resonance images, significant enhancement of the geniculate ganglion is observed.[26]

Pathological changes in the auditory nerve and labyrinth have been reported.[7,10,23] In all cases, the patients had vertigo and hearing loss associated with facial nerve paralysis. As long as 7 months after the acute facial paralysis, variable perineural, intraneural, and perivascular lymphocytic or round cell infiltration involving the facial nerve, auditory nerve, and particularly the vestibular nerve, as well as the modiolus and organ of Corti in the cochlea, was present.[7] Inflammatory cells have also been seen in the maculae of the utricle and saccule. In a specimen obtained 21 months after facial paralysis, the branch of the vestibular nerve that leads to the lateral and superior semicircular canals was atrophic.[10] Corresponding atrophy and loss of hair cells of the cristae of the corresponding semicircular canals were noted. In 1 case, collapse of Reissner's membrane was present. Furthermore, pathological changes in the brain stem, cerebellum, and cerebrum[27] were also reported.

In addition to histopathologic studies, molecular studies of VZV genomes have helped to further elucidate the mechanism of virus spread through the infected tissues. In patients with Ramsay Hunt syndrome, VZV DNA was detected in the middle ear mucosa around the facial canal, the auricular muscles, the endoneural fluid, and the nerve sheath of the facial nerve.[28] In another study,[29] VZV genomic DNA was found in sections of the geniculate ganglion, the spiral ganglion, Scarpa's ganglion, the organ of Corti, and the macula of the saccule. A possible route of infection of the vestibulocochlear components by VZV may be spread of the virus in the facial nerve to the eighth cranial nerve via the anastomotic communicating nerve branch, or even infection of the hair cells in the cochlea and vestibule through their windows from a virus in the middle ear mucosa.[28] However, latent infection of VZV has also been shown in human trigeminal, thoracic, and spiral ganglia, as well as the geniculate ganglion. Therefore, the possibility exists that the virus spreads from other ganglia or is simultaneously reactivated from the spiral ganglion. Also, in various studies[28-30] of patients with the disease, asymptomatic meningoencephalitis has been diagnosed by the presence of VZV DNA in the cerebrospinal fluid, increases in the titer of anti-VZV antibody, and increases in mononuclear cell number, which may be the cause of cranial neuropathy. The VZV genomes have been detected further in peripheral blood mononuclear cells and urine from patients with Ramsay Hunt syndrome.[31] Therefore, it is reasonable to accept that VZV migration in this syndrome is much more extensive and systematic than previously thought, although identification of VZV genomes does not always provide definitive evidence for the presence of infective virus.

From all of these pathological and molecular studies, evidence was provided that during inflammation caused by VZV, the extension of pathological changes beyond the geniculate ganglion is common in the vestibulocochlear nerve, the brain stem, the cerebellum, and the cerebrum. The diversity of these findings and the presence of asymptomatic meningoencephalitis, which is observed quite often in these patients, may explain the ABR abnormalities found in most of them, as desynchronization and poor neural conduction may account for the increased latencies and interlatencies observed. Cochlear involvement sometimes accompanies the retrocochlear lesions, but pure cochlear hearing loss is not as frequent.

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EVALUATION OF NASAL MUCOCILIARY CLEARANCE FUNCTION IN ALLERGIC RHINITIS PATIENTS WITH TECHNETIUM 99M–LABELED MACROAGGREGATED ALBUMIN RHINOSCINTIGRAPHY

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Nasal mucociliary clearance is the first line of defense of the ciliated epithelium of the respiratory tract against inhaled particles. Several rhinopathic conditions involve alterations in nasal mucociliary clearance. The purpose of this study was to assess the clinical role of rhinoscintigraphy in evaluation of nasal mucociliary clearance function in allergic rhinitis patients and in sinusitis patients and healthy controls for comparison. A technetium 99m–labeled macroaggregated albumin (Tc-99m MAA) drop was deposited on the floor of the nasal meatus about 1 cm behind the mucocutaneous junction, and its course was followed with a gamma camera. The nasal mucociliary clearance function was measured as the velocity of the nasal mucociliary transport of the Tc-99m MAA droplet. The mean velocity of nasal mucociliary transport in the allergic rhinitis patients was 2.71 ± 0.76 mm/min (2.65 ± 0.76 mm/min in the right nostril; 2.77 ± 0.76 mm/min in the left nostril). The mean velocity of nasal mucociliary transport in the sinusitis patients was 1.80 ± 0.64 mm/min (1.79 ± 0.65 mm/min in the right nostril; 1.82 ± 0.63 mm/min in the left nostril). The mean velocity of nasal mucociliary transport in the 25 healthy controls was 4.28 ± 1.38 mm/min (4.30 ± 1.35 mm/min in the right nostril; 4.27 ± 1.42 mm/min in the left nostril). A significant difference in nasal mucociliary clearance was observed among the 3 groups. We suggest that Tc-99m MAA rhinoscintigraphy may be a useful noninvasive tool to evaluate the nasal mucociliary clearance function in allergic rhinitis patients.

KEY WORDS — allergic rhinitis, rhinoscintigraphy, technetium 99m–labeled macroaggregated albumin.

Allergic rhinitis is characterized by an increase in the numbers of nasal mast cells and basophils.[1] The cardinal symptoms of allergic rhinitis are nasal obstruction, sneezing, and watery rhinorrhea. Allergic rhinitis can produce significant morbidity and is often costly to the health care system. The nasal mucosa is supplied with dense sensory innervation that is of importance for defense reactions to protect the airways against inhalation of irritant chemicals and particles in the air. Disturbances in nasal mucociliary clearance function are clearly a more common cause of long-term respiratory disease, nasal infection, sinusitis, rhinitis, and otitis media than is presently recognized. Various techniques have been used to evaluate the ciliary activity of the nasal mucosa and the frequency of ciliary beats, including radiography, stroboscopy, and photoelectrons, but all are unsuitable for routine clinical use.[2-4] It is desirable to examine nasal mucociliary function by a simple and reliable method. The aim of this study was to assess whether a radioisotope method with technetium 99m–labeled macroaggregated albumin (Tc-99m MAA) can play a significant role in evaluation of the ciliary activity of the nasal mucosa and the nasal mucociliary clearance function in patients with allergic rhinitis.

MATERIALS AND METHODS

Subjects. The study population consisted of 25 patients with allergic rhinitis (13 men and 12 women ranging in age from 28 to 76 years), 25 patients with sinusitis (12 men and 13 women ranging in age from 26 to 76 years) as disease-positive controls, and 25 healthy adults (14 men and 11 women ranging in age from 27 to 77 years). The healthy subjects had no history of anatomic, clinical, or radiographic signs of nasal or paranasal sinus disease. Those with allergic rhinitis had positive skin tests for common inhalant allergens and perennial nasal symptoms and/or signs in previous years. No subjects with a smoking history were included in this study.

Rhinoscintigraphy. One drop of Tc-99m MAA (15 µCi/15 µL) was placed under visual control, by means

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DETAILED SUBJECT DATA

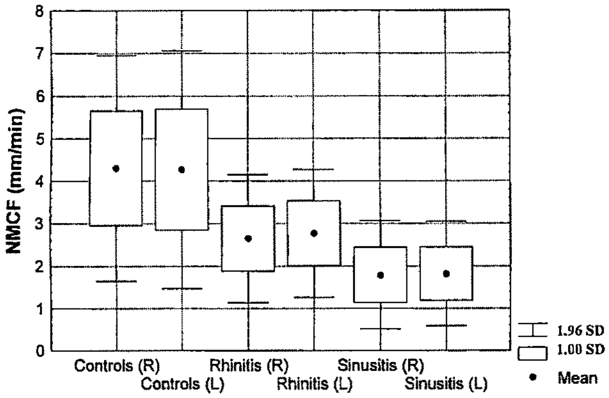
	Healthy Controls				*Allergic Rhinitis Patients*				*Sinusitis Patients*			
			NMCF (mm/min)				*NMCF (mm/min)*				*NMCF (mm/min)*	
Case No.	*Sex*	*Age (y)*	*Right*	*Left*	*Sex*	*Age (y)*	*Right*	*Left*	*Sex*	*Age (y)*	*Right*	*Left*
1	F	27	4.04	4.48	F	28	2.36	2.63	M	26	1.23	1.89
2	M	32	2.94	1.67	F	30	1.62	1.56	F	29	0.98	1.26
3	M	35	5.22	5.02	M	33	2.33	3.02	F	33	2.31	1.65
4	F	38	3.72	2.00	M	36	2.72	2.32	M	36	0.72	1.02
5	F	40	2.93	4.44	M	39	1.23	1.52	F	38	1.93	1.22
6	M	41	2.55	5.72	F	42	1.89	2.01	F	42	1.55	2.01
7	M	43	3.61	3.30	F	44	2.34	2.03	M	43	2.03	1.98
8	F	45	3.12	3.18	M	46	3.00	2.98	M	46	3.02	2.68
9	M	49	4.94	2.62	M	48	2.98	2.56	M	49	2.89	3.02
10	F	50	2.58	3.44	F	51	2.39	3.01	M	49	1.58	1.25
11	M	53	7.48	7.20	F	52	4.23	3.98	F	51	0.99	0.69
12	F	55	6.24	6.44	M	54	3.23	4.21	F	56	1.89	1.78
13	M	58	3.52	2.90	F	56	1.52	1.69	F	58	1.65	1.56
14	F	60	3.91	4.79	M	59	2.97	2.78	F	59	2.25	2.09
15	M	61	3.38	4.34	M	62	1.28	1.45	M	62	1.36	1.46
16	M	62	6.55	4.41	F	62	4.22	3.98	F	62	0.78	1.03
17	F	63	3.03	5.38	M	64	3.01	3.25	M	65	2.01	1.69
18	M	66	3.57	6.74	M	65	2.98	3.69	M	67	2.36	2.65
19	F	68	4.62	5.22	F	67	2.56	2.89	F	68	3.13	2.98
20	M	70	3.13	2.95	M	69	2.36	3.01	F	70	1.25	2.06
21	M	71	5.57	3.97	F	72	2.78	2.54	F	71	2.05	1.77
22	F	72	5.77	3.61	F	74	3.22	3.01	M	72	1.98	2.56
23	M	74	4.26	3.71	F	74	3.12	2.98	M	73	1.54	2.36
24	F	75	5.49	3.55	M	75	3.24	2.89	F	75	1.39	1.24
25	M	77	5.38	5.58	M	76	2.56	3.25	M	76	1.78	1.53
Mean		55.4	4.30	4.27		55.1	2.65	2.77		55	1.79	1.82
SD		14.7	1.35	1.42		15.0	0.76	0.76		15.3	0.65	0.63

NMCF — nasal mucociliary clearance function.

of a micropipette, on the floor of the nasal meatus about 1 cm behind the anterior end of the inferior turbinate. All tested subjects were in a supine position with the head laterally placed under a gamma camera and were advised to breathe normally through the nose or mouth and to avoid sniffing. The transport of the radiotracer was monitored by means of a gamma camera with a low-energy, all-purpose, parallel-hole collimator from either side of the head at 20 seconds per frame for a total of 30 minutes with a zoom 2, 64 × 64 word matrix. Two radioactive markers were placed at a distance of 100 mm with the number of pixels equal to 100 mm decided on the display images before the studies. The true distance of 1 pixel (millimeters per pixel) was then calculated. The total distance in millimeters of any pixel from the initial droplet in the display images could be calculated. On each of the regions of interest, the time-activity curves were established, and the frame number from the first frame on the peak of the time-activity curve was calculated and corresponded to the motion of the radiotracer. From these data, we could calculate the total time (minutes) needed for the radiotracer to arrive at the pixel from the initial pixel. By use of a least-square linear regression method, according to the distance (pixel numbers) and time (frame numbers) at which the tracer arrived at the pixel from the initial droplet, a new regression curve was established in which the slope (pixels per minute) of the curve represented the velocity (millimeters per minute).[5,6] The clearance velocity (centimeters per minute) of the Tc-99m MAA droplet was then calculated. All measurements in the 3 groups of subjects were taken in a controlled atmosphere at a temperature of 20°C ± 1°C and a relative humidity of 74% ± 2%. In this study, Student's *t*-tests were used for comparisons of data of the 3 groups of subjects.

RESULTS

The detailed results are shown in the Table. Among the 75 subjects, we observed no significant discomfort or side effects related to scintigraphy. The mean velocities of nasal mucociliary clearance of the 25 healthy adults, 25 allergic rhinitis patients, and 25 sinusitis patients were 4.28 ± 1.38 mm/min (4.30 ± 1.35 mm/min in the right nostril; 4.27 ± 1.42 mm/min in the left nostril), 2.71 ± 0.76 mm/min (2.65 ± 0.76 mm/min in the right nostril; 2.77 ± 0.76 mm/min in the left nostril), and 1.80 ± 0.64 mm/min (1.79 ± 0.65 mm/min in the right nostril; 1.82 ± 0.63 mm/



Distribution of nasal mucociliary clearance function (NMCF) results of healthy controls, patients with allergic rhinitis, and patients with sinusitis.

min in the left nostril), respectively (see Figure). A statistically significant difference in nasal mucociliary clearance was found among the 3 groups.

DISCUSSION

The nasal mucosa, with its rich vascularization and glandular tissue, plays an important role in the protection of the lower airways by conditioning the inspired air. The nasal mucociliary transport capacity is dependent on the propulsive movements of the cilia and the braking force of the secretions. Nasal mucociliary clearance represents one of the main aspecific defensive mechanisms of the respiratory tract.[7] The nasal mucociliary transport time is a reliable index of upper and lower respiratory tract clearance function. There is alteration of nasal mucociliary clearance function in various rhinopathic conditions, and several methods have been used to evaluate the efficiency of nasal mucous transport. In practice, monitoring the particles has proven to be difficult, and the velocities at different stages cannot be determined. In 1965, Proctor and Wagner[6] first described a technique using the radioisotope-labeled particle iodine 131 MAA to record the transit velocity of particles along the nasal tract. Subsequently, various radioisotopic methods were proposed for rhinoscintigraphy.[8-12] However, Tc-99m MAA is preferred by most authors. In this study, the mean velocity of nasal mucociliary clearance in our healthy control group (see Table) was slower than that in studies of populations of other ethnicities.[8-12] The speed of nasal mucociliary transport is highly variable, even in identical environmental conditions. In fact, consistent differences in nasal mucociliary transport velocity have been observed with different techniques. However, it is difficult to determine which test is the most suitable for measuring the real mucociliary function. The results of this study showed that allergic rhinitis patients have a prolonged mucociliary transit time as compared with healthy controls. The difference between normal controls and allergic rhinitis patients was significant. The fact that mucociliary clearance is decreased in allergic rhinitis is an interesting finding that may shed light on the pathogenesis of allergic rhinitis, although it may not have any immediate applicability to treatment.

In conclusion, Tc-99m MAA rhinoscintigraphy cannot be used for the diagnosis of allergic rhinitis, because several other disorders such as sinusitis can impair mucociliary clearance. However, it may be a useful noninvasive tool to evaluate the nasal mucociliary clearance function in allergic rhinitis patients. Because all measurements were taken under the same conditions (a temperature of 20°C ± 1°C and a relative humidity of 74% ± 2%), it was not necessary in our study to evaluate the effect of low humidity, as most new buildings have a relative humidity of less than 70% because of air conditioning. However, we suggest that future researchers should evaluate their controls and patients under the same conditions.

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LOCAL ANESTHESIA WITH EMLA CREAM FOR MAXILLARY SINUS PUNCTURE

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Maxillary sinus puncture is traditionally carried out through the lateral wall of the inferior nasal meatus under local anesthesia. One problem with it is that the insertion of a cotton-tipped applicator soaked in local anesthetic is painful. Patients also dislike waiting for the anesthetic effect with the metallic applicators in the nose. In this study, we present a new, well-tolerated method of topical anesthesia for maxillary sinus puncture via the inferior meatus of the nose. Twenty adult patients with maxillary sinus infection who were undergoing bilateral maxillary sinus puncture were studied. One side of each patient's nose was anesthetized with a cotton-tipped applicator moistened with a lidocaine-adrenaline solution (LA), and the other side was anesthetized with EMLA cream instilled with a suction needle and syringe; the sides were chosen randomly. The mean "application of anesthesia" pain score on a 100-mm visual analog scale was 39.2 for the LA side and 9.1 for the EMLA side ($p < .01$). The anesthesia required for puncture was reached more quickly on the EMLA side than on the LA side ($p = .02$). The mean puncture pain score was 25.1 with LA and 8.6 with EMLA ($p = .01$). Fourteen patients out of the 20 (70%) found EMLA more tolerable, 3 patients (15%) found no difference, and 3 patients (15%) preferred LA ($p < .01$). We conclude that EMLA is better-tolerated and quicker-acting than LA for local anesthesia in maxillary sinus puncture.

KEY WORDS — antral puncture, EMLA, local anesthesia, sinusitis, treatment.

INTRODUCTION

In experienced hands, maxillary sinus puncture with saline irrigation is a relatively simple and useful procedure for both the diagnosis and treatment of sinusitis.[1,2] In cases of acute empyema, patients usually feel immediate relief of sinus pain after this procedure. Also, this procedure makes it possible to obtain an adequate microbiological culture from maxillary sinus secretions.[3] The recent emergence of resistant bacteria increases the importance of sinus puncture even more, especially in patients who are immunosuppressed or patients who do not respond to empirical therapy.[4] However, the traditional form of local anesthesia with cotton-tipped probes soaked in an anesthetic solution is not well tolerated by patients, mainly because the insertion of the applicators is painful, and waiting for up to 20 minutes for the anesthetic effect of the probes in the nose is unpleasant.

EMLA, which is an oily mixture of lidocaine hydrochloride (25 mg/mL) and prilocaine hydrochloride (25 mg/mL), has been shown to be a relatively safe and effective topical anesthetic both on skin and on mucosal surfaces.[5,6] The purpose of this study was to investigate whether EMLA cream can be used for effective local anesthesia for maxillary sinus puncture and to compare the application technique of EMLA with the traditional technique.

PATIENTS AND METHODS

Twenty adult patients (12 male and 8 female) with maxillary sinus infection who were undergoing bilateral maxillary sinus puncture were studied. One side of each patient's nose was anesthetized by inserting a cotton-tipped applicator soaked in lidocaine-adrenaline solution (LA) composed of lidocaine hydrochloride (40 mg/mL) and epinephrine LE (1:1,000) into the inferior meatus of the nose, and the other side was anesthetized with a metallic suction needle (1.4 × 60 mm) connected to a 2-mL disposable plastic syringe containing EMLA cream (Fig 1). The sides were selected by using a random number table, and the right-hand side was always anesthetized first.

The suction needle was carefully inserted into the inferior meatus, and the piston was pressed until there was 1 mL of EMLA cream under the inferior turbinate (Fig 2). The suction needle was then withdrawn.

Immediately after the application of the anesthetic, the patients were asked to rate their pain on a 100-mm visual analog scale (VAS). The pain assessment was made on the first side before the local anesthetic was applied to the other side. The VAS score was recorded, and the scores for the LA side were compared to those for the EMLA side. A score of 0 rep-

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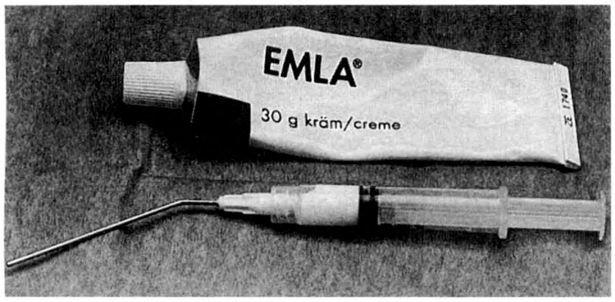

Fig 1. EMLA cream is applied with angled metallic suction needle (1.4 × 60 mm) connected to 2-mL disposable plastic syringe.

resented no pain, and a score of 100 represented the greatest possible pain.

On the LA side, 5 minutes after the insertion of a cotton probe soaked in LA, the probe was pushed against the mucosa and the patient was asked about his or her sensation of pain. This was repeated after every 5 minutes until the patient no longer felt pain. The time was recorded.

After a 5-minute application of EMLA, a plain, cotton-tipped probe was inserted for a short while into the inferior meatus and pushed toward the mucosa, and the patient was again asked to rate his or her pain. As on the LA side, this was repeated every 5 minutes until there was no pain sensation on either side.

Immediately after the puncture, the patient was asked to rate his or her sensation of pain on the VAS. The pain assessment on the first puncture side was made before a new puncture took place. Afterward, the maxillary sinus was irrigated with warm saline solution. The patients were finally asked which anesthesia method they found more tolerable.

The study was approved by the ethics committee of Tampere University Hospital. Statistical analysis was carried out with the Wilcoxon signed-rank test.

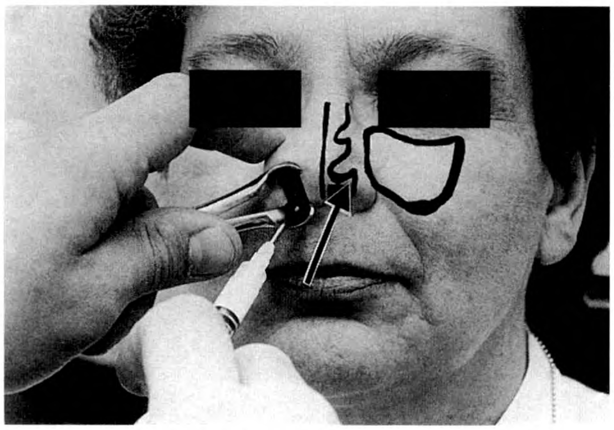

Fig 2. Suction needle is inserted into inferior meatus, and piston is pressed until there is 1 mL of EMLA cream under inferior turbinate.

TIME NEEDED TO REACH ANESTHESIA WITH EMLA AND LA

	EMLA		*LA*	
Time (min)	*No. of Pts.*	*%*	*No. of Pts.*	*%*
5	19	95	12	60
10	1	5	5	25
15			2	10
20			1	5

LA — lidocaine and adrenaline.

RESULTS

The mean pain scores for application of anesthesia were 39.2 for LA and 9.1 for EMLA ($p < .01$). When the applicator was pushed against the mucosa, the patients stopped feeling pain sooner on the EMLA side than on the LA side ($p = .02$; see Table). The mean puncture pain score was 25.1 for LA and 8.6 for EMLA ($p = .01$). Fourteen patients out of the 20 (70%) considered EMLA to be more tolerable, 3 patients (15%) found no difference, and 3 patients (15%) preferred LA ($p < .01$).

DISCUSSION

Although there are various routes for irrigation of the maxillary sinus,[7] the most commonly used is the inferior meatal route. Antral puncture is usually performed under local anesthesia. The traditional and most widely used method to obtain local anesthesia for puncture is to insert cotton-tipped probes soaked in anesthetic solution into the inferior meatus of the nose. This method, however, is not well tolerated by patients, since the insertion of the applicators is painful and it is unpleasant to wait for up to 20 minutes for the anesthetic effect with the probes in the nose. This unpleasant method of anesthesia decreases the willingness of many patients to undergo maxillary sinus puncture.

In the present study, most of the patients found EMLA more tolerable than LA. The application of EMLA cream was almost painless. EMLA was also significantly quicker-acting (see Table). Further, the anesthesia obtained with EMLA was better than that obtained with the LA solution. The superiority of the performance of EMLA cream to that of other topical anesthetics has been found by other authors as well.[8]

Cocaine solution, which has also been widely used as a local anesthetic in intranasal procedures, appears to be more effective than lidocaine.[9] However, serious side effects have been reported with the use of cocaine in topical anesthesia,[10-12] whereas EMLA has been shown to be relatively safe when applied to mucous membranes.[13]

Currently, most of the maxillary sinus punctures at our otorhinolaryngology department are performed with the EMLA method.

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FUNCTIONAL DEFINITIONS OF VOCAL FOLD GEOMETRY FOR LARYNGEAL BIOMECHANICAL MODELING

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Precise geometric data on vocal fold dimensions are necessary for defining the vocal fold boundaries with respect to the laryngeal framework in physiological and biomechanical models of the larynx (eg, finite-element models). In the mid-membranous coronal section, vocal fold depth can be defined as the horizontal distance from the vocal fold medial surface to the thyroid cartilage, whereas vocal fold thickness can be defined as the vertical distance from the inferior border of the thyroarytenoid muscle to the vocal fold superior surface. Traditionally, such geometric data have been obtained from measurements made on histologic tissue sections. Unfortunately, it is very difficult to obtain reliable data by this method, unless the effects of sample preparation on vocal fold geometry are quantified. Significant tissue deformations are often induced by histologic processes such as fixation and dehydration, sometimes producing shrinkages as large as 30%. In this study, reliable geometric data of the canine vocal fold were obtained by the alternative method of quick-freezing for sample preparation, using liquid nitrogen. Coronal sections of quick-frozen larynges were thawed gradually in saline solution. Images of the mid-membranous coronal sections at various thawing stages were captured by a digital camera. Measurements of operationally defined vocal fold dimensions (depth and thickness) useful for biomechanical modeling were made with a graphics software package. The results showed that geometric changes of the vocal fold induced by freezing are likely reversed by thawing, such that the measurements made on thawed larynges are reliable approximations of the actual vocal fold dimensions.

KEY WORDS — biomechanical modeling, quick-freezing, thickness, vocal fold depth, vocal fold geometry.

INTRODUCTION

In the study of voice production, excised larynges from humans and animals have often been used. The canine larynx has frequently been used because of its relative ease of acquisition and its similarities in size and in basic anatomy with the human larynx. For instance, active contractile properties and passive stress-strain characteristics of the canine intrinsic laryngeal muscles have been measured previously[1-3] (also Tayama et al, unpublished data). These data are important for the physiological and biomechanical modeling of voice production. Geometric data on the larynx are also important information for biomechanical modeling, including the 3-dimensional orientations of the intrinsic laryngeal musculature,[4] as well as the dimensions of the laryngeal cartilage framework[5] and the vocal fold. The mechanics of phonation are critically dependent on the geometry of the vocal folds. For example, it has been shown that phonation threshold pressure, the minimum subglottal pressure required to initiate and sustain vocal fold oscillation, is dependent on the thickness of the vocal fold and the shape (convergence or divergence) of the glottis.[6] Differences in vocal fold geometry between subjects of different age and gender, and between humans and other species, are also valuable information for studies of their differences in voice production.

Vocal fold structure is often discussed on the basis of the body-cover theory.[7] Previous studies have reported geometric data on the cover, which is the major vibratory portion of the vocal fold.[8-10] From histologic tissue sections, Hirano et al[11,12] reported that the "thickness" of the vocal fold cover was 1.0 to 1.5 mm in the human larynx. Kurita et al[13] measured the "thickness" of the vocal fold mucosal layer in humans and in different animals, including dogs. They reported that it was around 3 mm in dogs. From grossly dissected canine vocal folds, Perlman and Durham[14] reported the "thickness" of the cover to be

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TABLE 1. SUBJECT INFORMATION

Canine Subject	*Sex*	*Weight (kg)*
C1	M	20
C2	M	20
C3	M	20
C4	M	27
C5	M	22
C6	M	30
C7	F	25
C8	M	27
C9	F	23
C10	M	20
C11	F	19
C12	F	20
C13	F	21
C14	M	23
C15	F	25

around 2.5 mm in male dogs and 2.0 mm in female dogs.

In the above studies, the "thickness" of the vocal fold measured was not well defined geometrically, and the "depth" of the vocal fold was not measured. Therefore, these data are not optimal for the purpose of biomechanical modeling, which requires precisely defined measurements positioned in a coordinate system with respect to the laryngeal cartilage framework. For example, the vocal fold "thickness" in those studies was only measured at 1 point, and it was measured arbitrarily (in an oblique direction[11-13]), rather than in a well-defined coordinate system. A larger number of data points are needed to express more realistically the geometric contour of the vocal fold, but not so many data points that they would compromise the computational efficiency of the biomechanical models.

There are also methodological problems associated with the measurements of vocal fold geometry in the above studies. The vocal folds are made of soft tissues. In many studies, geometric measurements have been made on the mid-membranous coronal section of the vocal fold prepared histologically. Unfortunately, geometric deformations of different types of soft tissues are commonly observed during histologic sample preparation processes such as formalin fixation and dehydration. For example, shrinkages of human oral tissues as large as 30% have been reported.[15] The above reports on vocal fold dimensions did not consider these possible tissue shrinkages.

To minimize tissue deformation, Eckel and Sittel[16] measured the geometry of the human larynx in horizontal tissue sections using the alternative method of quick-freezing for sample preparation, instead of using histologic methods. Quick-freezing with liquid nitrogen has been shown to induce only minimal changes in the dynamic mechanical properties of vocal fold tissues.[17] It has also been commonly used for tissue sample preparation in electron microscopy[18] and in tissue culture.[19] Even with quick-freezing, however, there could still be some tissue deformation (eg, about 6% expansion in human head and neck muscles[20]). Still, quick-freezing is probably the best technique for the preservation of soft tissues, and causes only minimal changes in geometry. Although frozen tissues would expand in volume, the deformation is considerably less than that caused by histologic preparation methods.[16,18] The rate of deformation is different in different types of tissues with different processing methods. If we could quantify how much the vocal fold deforms during quick-freezing and across different stages of thawing, the actual dimensions of the vocal fold in vitro could be approximated.

This study attempted to operationally define the "depth" and "thickness" of the vocal fold with respect to the laryngeal cartilage framework, in order to efficiently represent the vocal fold geometry in biomechanical models of the larynx (eg, finite-element models[21]). We attempted to obtain reliable geometric data on the canine vocal folds by comparing frozen and thawed larynges. Canine tissues were used because they could be obtained immediately after death, while it was practically not possible to obtain fresh-enough human larynges. It was important to use fresh tissues so that the stages of rigor mortis would not induce significant tissue deformation before the vocal fold geometry could be preserved by quick-freezing. The geometric data on the canine vocal fold obtained in the present study are useful for functionally defining the vocal fold boundaries with respect to the laryngeal framework in biomechanical models of the larynx.

METHOD

Subjects. Fifteen canine larynges were excised within 10 minutes of death after cardiovascular experimentation and euthanasia of the animals, in accordance with the Institutional Animal Care and Use Committee of the University of Iowa. All specimens were obtained from adult mongrel dogs without evidence of laryngeal trauma or head and neck diseases (see Table 1 for subject information). No animals were sacrificed specifically for the present investigation.

Sample Preparation. For each larynx, soft tissues superior to the true vocal folds were removed, including the epiglottis, aryepiglottic folds, and false vo-

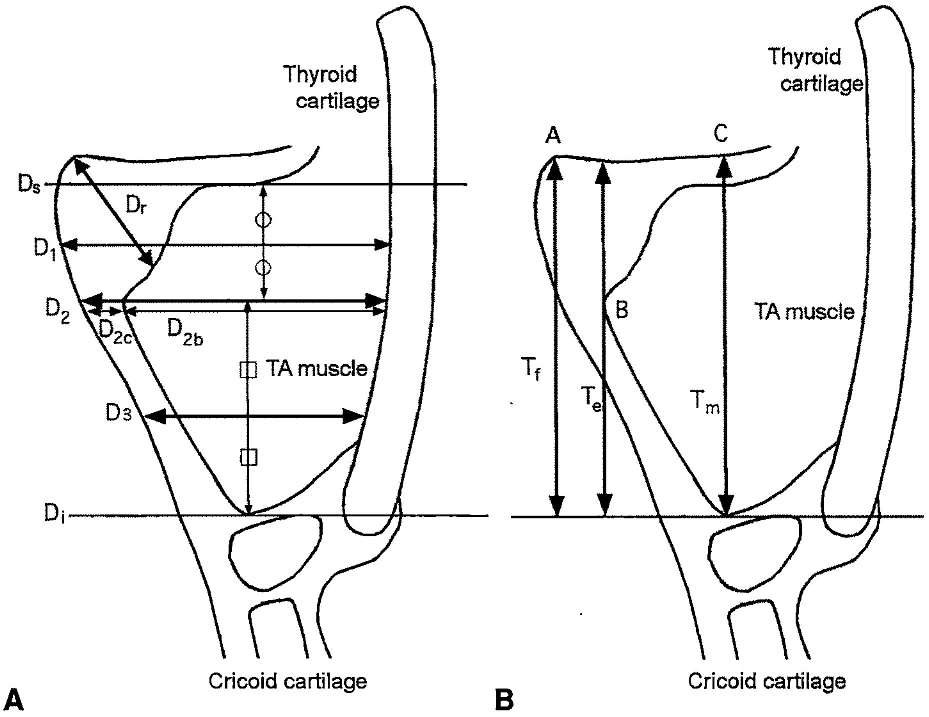

Fig 1. Schematic illustrations of operational definitions of **A**) vocal fold depth and **B**) vocal fold thickness in mid-membranous coronal section. See text for definitions.

cal folds. In order to measure vocal fold length, we made marks on the anterior commissure, the mid-membranous point, and the vocal process of each vocal fold with black India ink before freezing. The larynx was quickly frozen by chilling in liquid nitrogen (boiling point, −196°C). It was mounted by securing the trachea over a piece of polyvinyl chloride tube with an O-clamp around the first and second tracheal rings. Other clamps were also used to hold the thyroid and cricoid cartilages in order to securely fix the larynx. The vocal fold length of 10 larynges (C1 to C10), defined as the distance between the anterior commissure and the vocal process, was measured by a digital caliper before and after freezing. It was also measured after thawing for 5 of the 10 larynges (C1 to C5). In order to measure vocal fold depth and thickness, 5 larynges (C11 to C15) were cut at the mid-membranous point in the coronal plane while they were still frozen, by means of a custom-built microtome. Thawing of the larynges was carried out in saline solution at room temperature (20°C). Images of the larynges in the mid-membranous coronal section were captured at the following times for subsequent geometric measurements: 1) during the frozen stage and 2) every 30 minutes during thawing, for up to 3 hours.

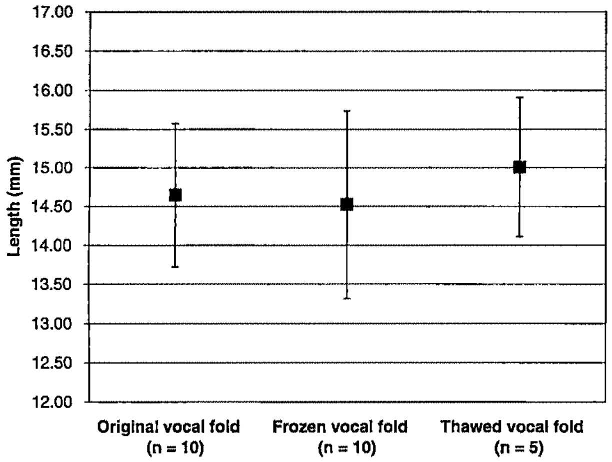

Fig 2. Vocal fold length changes with quick-freezing and thawing (mean length ± SD).

Image Acquisition. Scaled images of the vocal fold in the mid-membranous coronal section were captured with a digital camera (Ricoh, DC-3Z) with a spatial resolution of 640 × 480 pixels. The length resolution was 0.052 to 0.056 mm. The distortion of the digital images was less than 2% with the camera set at the condensed area mode. Image data in JPEG format were transferred to a computer, on which measurements of vocal fold depth and thickness were made with a graphic software package (Adobe Photoshop).

Definitions of Geometric Measurements. In the mid-membranous coronal section, the depth of the vocal fold (D) was defined as the distance from the medial surface of the vocal fold to the thyroid cartilage on a horizontal line, as shown schematically in Fig 1A. As this distance changes along the superior-inferior direction, it was defined at 5 different levels (Ds, Di, D1, D2, and D3) that can be clearly and consistently identified across larynges. Ds was defined at the level of the superior border of the thyroarytenoid (TA) muscle, while Di was defined at the level of the inferior border of the TA muscle. D_2 was defined at the level at which the medial edge of the TA muscle suddenly changes its curvature, such that the mucosal layer of the vocal fold (cover) becomes increasingly thicker superior to this level. D_1 was arbitrarily defined at a level equidistant to both D_s and D_2, whereas D_3 was defined at a level equidistant to both D_2 and D_i. In addition, each depth was also divided into 2 portions, one being the depth of the mucosal layer (the cover, D_c) and the other being the depth of the muscular layer (the body, D_b). Such definitions provide an efficient and functional representation of the vocal fold contour for biomechanical modeling. For comparison with previous studies, a reference "depth," D_r, is also shown in Fig 1A, which was defined in an arbitrarily oblique direction similar to the method of Hirano et al[11,12] and Kurita et al.[13]

TABLE 2. CHANGES OF VOCAL FOLD LENGTH AS RESULT OF QUICK-FREEZING AND THAWING

Subject	*Original Vocal Fold*	*Frozen Vocal Fold*	*Thawed Vocal Fold (at 2 h)*
C1	14.74	14.57	15.06
C2	13.42	12.91	13.48
C3	14.65	16.38	15.54
C4	15.24	15.62	15.21
C5	15.41	14.87	15.76
C6	16.32	15.54	MD
C7	14.67	13.35	MD
C8	13.54	12.79	MD
C9	13.57	14.23	MD
C10	14.93	15.00	MD
Mean	14.65	14.53	15.01
SD	0.93	1.21	0.90

Data are in millimeters. MD — missing data.

Vocal fold thickness was defined as the distance from the inferior border of the TA muscle to the vocal fold superior surface on a vertical line (Fig 1B). It was defined at 3 specific points (A, B, and C) that can be consistently identified across larynges, in order to capture the change of the vocal fold contour

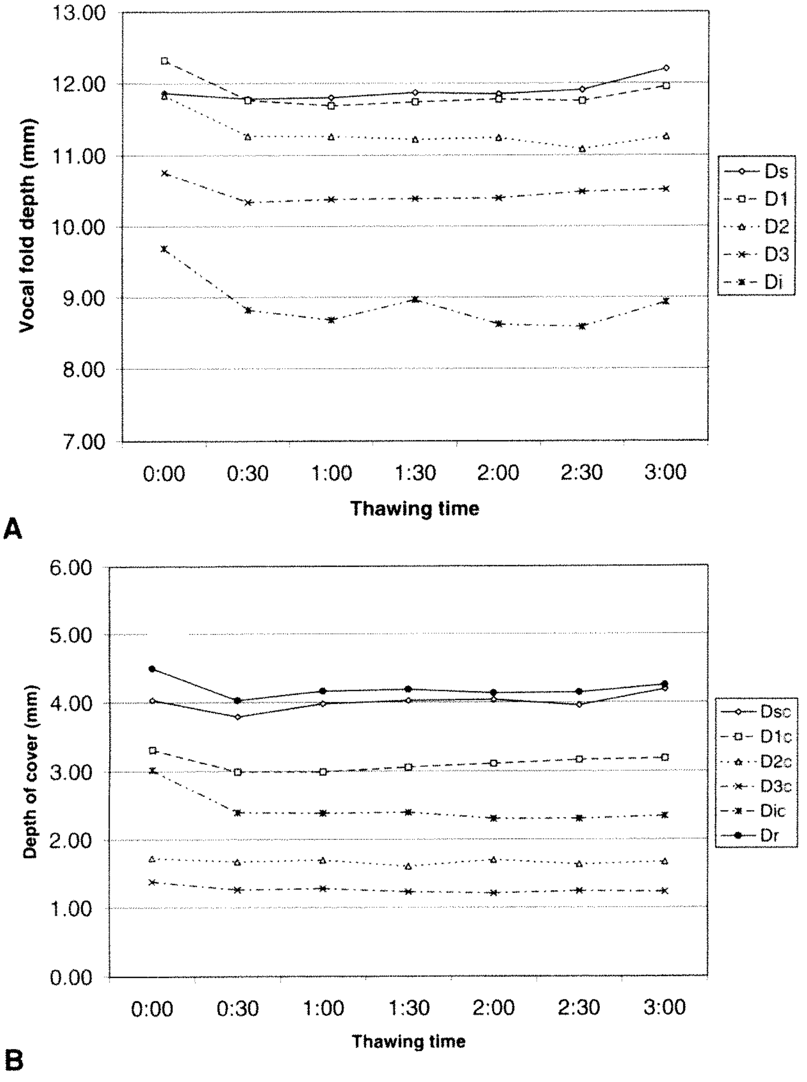

Fig 3. Mean vocal fold measurements as function of thawing time (n = 5 in each Figure). See text for definitions. **A)** Total depth. **B)** Depth of cover. (Continued on next page.)

along the medial-lateral direction: Tf was defined at the vocal fold free edge (point A: the "free-edge thickness"); Te was defined at the medial border of the TA muscle (point B: the "effective thickness"), at the level at which it suddenly changes its curvature (as in the definition of D2); and Tm was defined at the mucosal surface vertically above the inferior border of the TA muscle (point C: the "mucosal-muscle thickness").

These definitions of vocal fold depth and thickness emphasize the physiological and functional importance of the TA muscle in the control of vocal fold geometry, as it has been demonstrated that contraction of the TA muscle tends to bulge out the inferior portion of the vocal fold medially, creating a deeper and thicker vocal fold with a more rectangular glottal shape.[22] Furthermore, many of the definitions are based on the level at which the medial border of the TA muscle suddenly changes its curvature (point B in Fig 1B), which has previously been identified to be the point of mucosal wave upheaval.[23] Thus, the vocal fold depth D2 and the effective thickness Te, defined directly with respect to this point, should effectively capture the dimensions of the vibratory portion of the vocal fold. Data based on these definitions of vocal fold depth and thickness provide an efficient and functional representation of the vocal fold boundaries with respect to the laryngeal framework for biomechanical modeling applications.

RESULTS AND DISCUSSION

Vocal Fold Length. Figure 2 shows the changes of vocal fold length associated with the quick-freezing and thawing processes. Length measurements were obtained for 10 subjects (C1 to C10) before freezing (original in situ length) and after freezing (frozen length). Data were also obtained after 2 hours of thawing for 5 of the 10 subjects (C1 to C5). Because coronal sections were prepared for the other 5 subjects (C6 to C10) while the larynges were still frozen, length measurements made after thawing were not available for them. The average length of the vocal folds for the 10 subjects (C1 to C10) was 14.65 mm before freezing and 14.53 mm after freezing (ap-

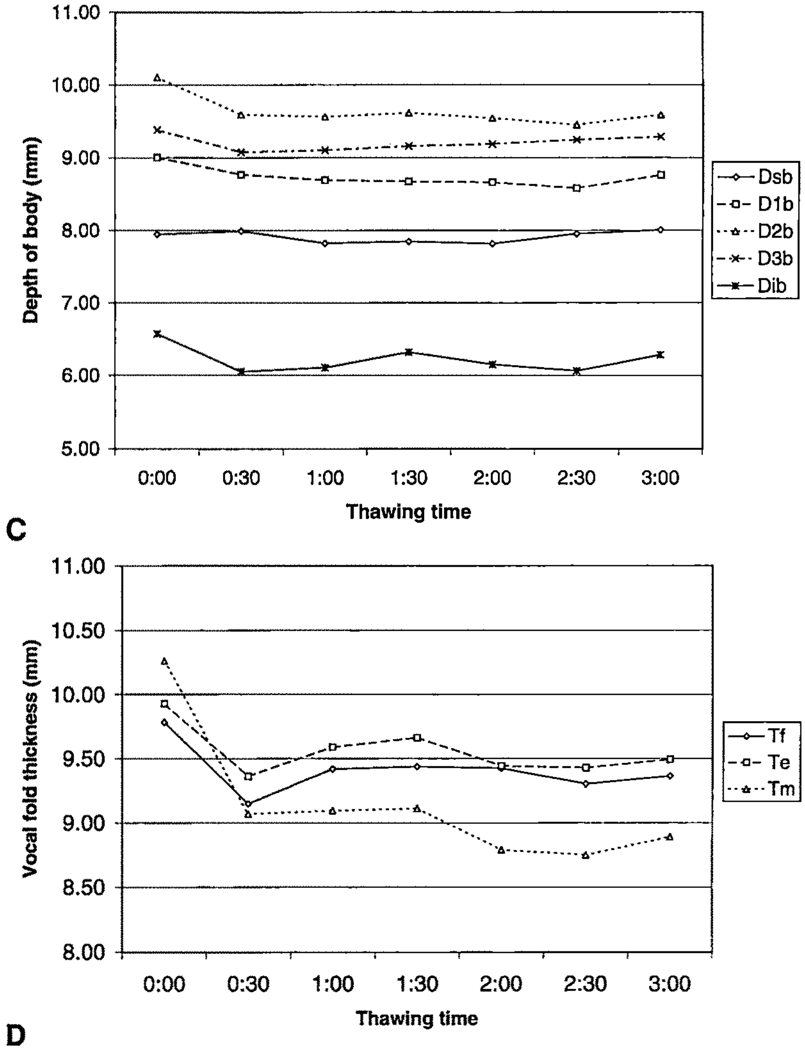

Fig 3, continued. **C**) Depth of body. **D**) Thickness.

TABLE 3. CHANGES OF TOTAL VOCAL FOLD DEPTH ACROSS FROZEN AND THAWING STAGES

			Thawing Time					
	Subject	*Frozen*	*30 min*	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
D_1	C11	11.33	11.18	11.11	11.33	11.22	11.40	11.42
	C12	10.97	9.99	10.14	10.67	10.21	10.31	11.11
	C13	12.15	12.21	11.68	11.82	12.07	12.11	12.00
	C14	13.10	12.38	12.50	12.18	12.03	11.78	11.85
	C15	14.05	13.06	13.02	12.70	13.36	13.17	13.40
	Mean	12.32	11.76	11.69	11.74	11.78	11.75	11.96
	SD	1.27	1.20	1.14	0.78	1.16	1.04	0.88
D_2	C11	11.01	10.54	10.48	10.71	10.59	10.67	10.72
	C12	10.72	10.26	10.57	10.84	10.44	10.46	11.05
	C13	11.80	11.56	10.92	10.86	10.94	10.96	10.87
	C14	12.51	11.70	12.07	11.71	11.52	11.01	11.22
	C15	13.11	12.27	12.27	11.98	12.72	12.32	12.43
	Mean	11.83	11.27	11.26	11.22	11.24	11.08	11.26
	SD	1.00	0.84	0.85	0.58	0.93	0.72	0.68
D_3	C11	10.36	9.48	9.48	9.56	9.40	9.77	9.75
	C12	10.36	9.88	9.99	10.24	9.91	9.77	10.44
	C13	10.76	10.97	10.92	11.09	11.58	11.94	11.19
	C14	10.95	10.75	10.60	10.52	10.15	10.22	10.17
	C15	11.38	10.62	10.91	10.55	10.94	10.74	11.03
	Mean	10.76	10.34	10.38	10.39	10.40	10.49	10.52
	SD	0.43	0.63	0.63	0.56	0.86	0.90	0.60
D_i	C11	8.98	7.98	7.58	7.79	7.48	7.48	7.68
	C12	10.40	9.67	9.78	10.14	9.76	9.69	10.18
	C13	MD	MD	MD	MD	MD	MD	MD
	C14	MD	MD	MD	MD	MD	MD	MD
	C15	MD	MD	MD	MD	MD	MD	MD
	Mean	9.69	8.82	8.68	8.97	8.62	8.58	8.93
	SD	1.00	1.19	1.55	1.66	1.61	1.56	1.77
D_s	C11	11.82	11.35	11.59	11.48	11.39	11.71	11.95
	C12	10.40	9.35	9.25	9.79	9.66	9.59	10.28
	C13	11.96	12.58	12.16	12.20	12.24	12.73	12.88
	C14	13.29	12.63	12.82	12.80	12.57	12.16	12.34
	C15	MD	13.00	13.19	13.08	13.40	13.35	13.55
	Mean	11.86	11.78	11.80	11.87	11.85	11.91	12.20
	SD	1.18	1.49	1.55	1.31	1.42	1.44	1.23

Data are in millimeters. See text for various definitions of total depth D. MD — missing data.

proximately a 1% decrease). For the 5 subjects in which measurements were also made after 2 hours of thawing (C1 to C5), the average vocal fold length was 14.69 mm before freezing, 14.87 mm after freezing (approximately a 1% increase), and 15.01 mm after thawing (another 1% increase). Table 2 shows the individual data for vocal fold length.

These results suggested that the magnitude of 1-dimensional changes in vocal fold tissue geometry as a result of quick-freezing and thawing was minimal (ranging from −1% to 2%) and was probably within the limits of measurement errors. It seemed that the method of quick-freezing, which has been commonly used for tissue sample preparation in electron microscopy and in tissue culture, indeed resulted in considerably less tissue deformation than that induced by histologic techniques of sample preparation (as much as 30% shrinkage).[15]

Vocal Fold Depth. Figure 3A-C shows the changes of vocal fold depth as a function of freezing and thawing, in terms of the different definitions of vocal fold depth illustrated in Fig 1A. Figure 3A shows the total depth D (body plus cover), which varied from approximately 8.6 to 11.8 mm along the superior-inferior direction across the thawing stages. The average depth D_s was 11.86 mm in the frozen stage and was 11.78 to 12.20 mm across the thawing stages (99% to 103% of the frozen dimensions). The average D_1 was 12.32 mm in the frozen stage and was 11.69 to 11.96 mm across the thawing stages (95%

TABLE 4. CHANGES OF VOCAL FOLD DEPTH OF COVER ACROSS FROZEN AND THAWING STAGES

		Thawing Time						
	Subject	*Frozen*	*30 min*	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
D_{1c}	C11	3.38	3.10	3.11	3.06	3.00	3.04	3.15
	C12	3.27	2.29	2.24	2.55	2.36	2.59	2.66
	C13	3.34	3.53	3.54	3.61	3.66	3.78	3.68
	C14	2.41	2.16	2.16	2.17	2.42	2.48	2.45
	C15	4.17	3.89	3.91	3.90	4.12	3.95	4.02
	Mean	3.31	2.99	2.99	3.06	3.11	3.17	3.19
	SD	0.63	0.76	0.78	0.72	0.77	0.67	0.66
D_{2c}	C11	2.03	2.18	2.05	2.08	2.15	2.15	2.13
	C12	1.61	1.64	1.82	1.67	1.91	1.72	1.88
	C13	1.45	1.27	1.10	1.07	1.04	1.09	1.13
	C14	1.24	1.10	1.24	1.03	1.06	0.90	0.89
	C15	2.31	2.16	2.28	2.17	2.35	2.32	2.32
	Mean	1.73	1.67	1.70	1.60	1.70	1.63	1.67
	SD	0.44	0.50	0.51	0.54	0.61	0.63	0.63
D_{3c}	C11	1.39	1.50	1.48	1.40	1.29	1.35	1.27
	C12	1.66	1.49	1.65	1.73	1.72	1.76	1.83
	C13	0.88	0.85	0.71	0.78	0.81	0.81	0.81
	C14	1.21	1.00	1.07	0.88	0.85	0.95	0.94
	C15	1.76	1.46	1.46	1.35	1.38	1.33	1.30
	Mean	1.38	1.26	1.28	1.23	1.21	1.24	1.23
	SD	0.36	0.31	0.38	0.39	0.39	0.37	0.40
D_{ic}	C11	3.04	2.62	2.15	2.34	2.24	2.15	2.34
	C12	3.20	2.92	3.00	2.96	2.70	2.90	2.96
	C13	2.57	1.82	1.96	2.03	1.80	1.58	1.56
	C14	3.36	2.62	2.65	2.69	2.74	2.83	2.82
	C15	2.93	2.00	2.17	1.98	2.05	2.07	2.03
	Mean	3.02	2.40	2.39	2.40	2.31	2.30	2.34
	SD	0.30	0.47	0.43	0.42	0.41	0.56	0.57
D_{sc}	C11	4.54	4.55	4.85	4.88	4.60	4.39	4.58
	C12	1.86	1.55	1.50	1.67	1.81	1.72	1.83
	C13	5.15	5.08	5.29	5.39	5.25	5.51	5.63
	C14	4.12	3.78	3.79	4.00	4.20	3.83	4.56
	C15	4.50	4.00	4.46	4.19	4.31	4.33	4.36
	Mean	4.04	3.79	3.98	4.03	4.04	3.96	4.19
	SD	1.27	1.35	1.49	1.43	1.31	1.39	1.41
D_r	C11	4.09	3.86	4.24	4.24	3.84	4.07	4.26
	C12	3.52	2.80	2.75	2.72	2.91	2.79	2.89
	C13	5.76	4.91	5.32	5.31	5.27	5.25	5.36
	C14	3.90	4.08	4.06	4.13	4.13	4.05	4.00
	C15	5.23	4.49	4.46	4.55	4.53	4.59	4.76
	Mean	4.50	4.03	4.17	4.19	4.14	4.15	4.26
	SD	0.95	0.79	0.93	0.94	0.87	0.91	0.92

Data are in millimeters. See text for various definitions of depth of cover D_c.

to 97% of the frozen dimensions). The average D_2 was 11.83 mm in the frozen stage and was 11.08 to 11.27 mm across the thawing stages (94% to 95% of the frozen dimensions). For D_3, the average was 10.76 mm in the frozen stage and was 10.34 to 10.52 mm across the thawing stages (96% to 98% of the frozen dimensions). For D_i, the average vocal fold depth was 9.69 mm in the frozen stage and was 8.58 to 8.97 mm across the thawing stages (89% to 93% of the frozen dimensions). In summary, it can be seen that the average vocal fold depth in the frozen stage was generally larger than that during thawing, which was almost steady over the entire course of thawing (for 3 hours). These data suggested that some tissue expansion (in most cases less than 5%) was likely induced by the liquid nitrogen quick-freezing process, and was effectively reversed by the thawing process.

TABLE 5. CHANGES OF VOCAL FOLD DEPTH OF BODY ACROSS FROZEN AND THAWING STAGES

			Thawing Time					
	Subject	*Frozen*	*30 min*	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
D_{1b}	C11	7.95	8.09	8.00	8.26	8.22	8.36	8.27
	C12	7.70	7.70	7.90	8.12	7.84	7.72	8.45
	C13	8.80	8.68	8.13	8.22	8.42	8.33	8.32
	C14	10.69	10.23	10.34	10.00	9.62	9.31	9.39
	C15	9.87	9.16	9.11	8.80	9.24	9.22	9.38
	Mean	9.00	8.77	8.70	8.68	8.67	8.59	8.76
	SD	1.27	0.99	1.04	0.79	0.74	0.67	0.57
D_{2b}	C11	8.98	8.36	8.44	8.63	8.43	8.52	8.59
	C12	9.11	8.61	8.75	9.16	8.54	8.74	9.17
	C13	10.35	10.29	9.82	9.79	9.90	9.87	9.73
	C14	11.27	10.60	10.83	10.68	10.47	10.11	10.33
	C15	10.81	10.10	9.99	9.81	10.37	10.00	10.11
	Mean	10.10	9.59	9.56	9.62	9.54	9.45	9.59
	SD	1.02	1.03	0.97	0.77	0.99	0.76	0.71
D_{3b}	C11	8.97	7.98	8.00	8.16	8.12	8.42	8.48
	C12	8.70	8.39	8.34	8.51	8.19	8.01	8.61
	C13	9.88	10.12	10.21	10.31	10.77	11.13	10.38
	C14	9.74	9.75	9.52	9.64	9.30	9.26	9.23
	C15	9.62	9.16	9.45	9.19	9.56	9.41	9.73
	Mean	9.38	9.08	9.10	9.16	9.19	9.25	9.29
	SD	0.52	0.90	0.91	0.86	1.10	1.20	0.79
D_{ib}	C11	5.94	5.36	5.43	5.45	5.24	5.34	5.34
	C12	7.20	6.75	6.78	7.18	7.06	6.79	7.22
	C13	MD	MD	MD	MD	MD	MD	MD
	C14	MD	MD	MD	MD	MD	MD	MD
	C15	MD	MD	MD	MD	MD	MD	MD
	Mean	6.57	6.05	6.11	6.32	6.15	6.06	6.28
	SD	0.89	0.98	0.95	1.23	1.29	1.03	1.33
D_{sb}	C11	7.27	6.80	6.73	6.60	6.78	7.32	7.36
	C12	8.54	7.80	7.75	8.12	7.86	7.87	8.45
	C13	6.80	7.49	6.87	6.81	6.99	7.22	7.25
	C14	9.17	8.86	9.03	8.80	8.36	8.32	7.77
	C15	MD	9.00	8.73	8.89	9.09	9.02	9.19
	Mean	7.94	7.99	7.82	7.84	7.82	7.95	8.01
	SD	1.10	0.93	1.05	1.08	0.96	0.74	0.81

Data are in millimeters. See text for various definitions of depth of body D_b. MD — missing data.

Figure 3B shows the various definitions of the depth of the cover (D_c), which varied from approximately 1.2 to 4.0 mm along the superior-inferior direction. The smallest depth of the cover was observed for D_{3c} (around 1.2 mm), while the largest depth was observed for D_{sc} (around 4.0 mm, not including the reference depth D_r). The obliquely defined cover depth (D_r) reported by Hirano et al[11,12] and Kurita et al[13] was around 3.0 mm in dogs, whereas in our measurements it was around 4.2 mm. Figure 3C shows the various definitions of the depth of the body (D_b), which varied from approximately 6.0 to 9.6 mm along the superior-inferior direction. D_{ib} was the smallest, because it did not include a significant muscle portion, while D_{2b} was the largest, because it involved the deepest TA muscle. Most of the data for the cover depth (D_c) and the body depth (D_b) changed with quick-freezing and thawing in a way very similar to that of the total vocal fold depth (D), as evidenced by the similar shapes of the curves in Fig 3A-C; ie, the average depth in the frozen stage was generally larger, but it became smaller and remained very stable across the thawing stages. Tables 3-5 show the individual data for vocal fold depth.

Vocal Fold Thickness. Figure 3D shows the changes of vocal fold thickness across the frozen and thawing stages, in terms of the various definitions of vocal fold thickness (T) illustrated in Fig 1B. The thickness varied from approximately 8.8 to 9.6 mm along the medial-lateral direction across the thawing stages. The average free-edge thickness (T_f) was 9.78 mm in frozen larynges and was 9.15 to 9.44 mm across the thawing stages (94% to 96% of the frozen dimen-

TABLE 6. CHANGES OF VOCAL FOLD THICKNESS ACROSS FROZEN AND THAWING STAGES

			Thawing Time					
	Subject	*Frozen*	*30 min*	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
Tf	C11	8.88	7.92	8.75	8.74	8.39	8.46	8.59
	C12	8.07	8.03	8.07	8.02	8.23	8.06	8.41
	C13	11.23	10.42	10.86	11.07	10.99	10.85	10.59
	C14	10.40	10.48	10.40	10.56	10.53	10.41	10.33
	C15	10.35	8.89	9.02	8.80	8.99	8.73	8.90
	Mean	9.78	9.15	9.42	9.44	9.43	9.30	9.36
	SD	1.28	1.25	1.17	1.31	1.26	1.24	1.02
Te	C11	9.09	8.13	8.90	8.99	8.34	8.52	8.76
	C12	7.86	8.18	8.24	8.33	8.29	8.06	8.41
	C13	11.49	10.76	11.07	11.25	10.83	11.06	10.93
	C14	10.55	10.64	10.61	10.77	10.57	10.62	10.37
	C15	10.66	9.10	9.13	8.99	9.18	8.87	9.00
	Mean	9.93	9.36	9.59	9.66	9.44	9.43	9.49
	SD	1.44	1.28	1.20	1.27	1.21	1.33	1.10
Tm	C11	9.41	7.92	8.23	8.57	7.69	7.78	8.38
	C12	9.48	8.83	8.66	8.33	7.69	7.68	7.89
	C13	11.49	10.48	10.53	10.69	10.49	10.53	10.33
	C14	9.98	9.07	8.99	9.12	9.05	9.00	8.96
	C15	10.97	9.06	9.08	8.85	9.03	8.77	8.90
	Mean	10.26	9.07	9.10	9.11	8.79	8.75	8.89
	SD	0.93	0.92	0.87	0.93	1.17	1.15	0.92

Data are in millimeters. See text for various definitions of thickness T.

sions). The average effective thickness (Te) was 9.93 mm in the frozen stage and was 9.36 to 9.66 mm during thawing (94% to 97% of the frozen dimensions). The average mucosal-muscle thickness (Tm) was 10.26 mm in the frozen stage and was 8.75 to 9.11 mm across the thawing stages (85% to 89% of the frozen dimensions). Similar to the data on vocal fold depth (Fig 3A-C), it can be seen that the average vocal fold thickness was generally larger while the tissues were still frozen, but became smaller and remained relatively stable over the course of thawing (for 3 hours). Again, these data suggested that the quick-freezing process likely induced some vocal fold tissue expansion (in most cases, within 5% to 10%), but such expansion was basically reversed by thawing. Table 6 shows the individual data for vocal fold thickness.

In summary, the findings of the present study support the hypothesis that there are only minimal 3-dimensional changes in vocal fold tissue geometry as a result of quick-freezing and thawing, in terms of vocal fold length (<2%), depth (<5%), and thickness (5% to 10%). These magnitudes of tissue geometric deformation are considerably smaller than those induced by histologic techniques of tissue sample preparation (as much as 30%).[15] Hence, it seems that the method of quick-freezing, which has already been commonly used for tissue sample preparation in electron microscopy and in tissue culture, is probably the better technique for the preservation and estimation of the in situ geometry of the vocal fold and other soft tissues in vitro. Figure 4 shows such an estimation of the reconstructed in situ vocal fold geometry based on the data of vocal fold depth and thickness averaged over the thawing process, as data from the thawing stages were good approximations of the prefreezing geometry.

Physiologically, the geometry of the vocal fold is heavily influenced by the activity of the TA muscle. Vocal folds with no or minimal TA muscle contraction tend to be thinner and have a more convergent glottal shape, while those with significant TA muscle

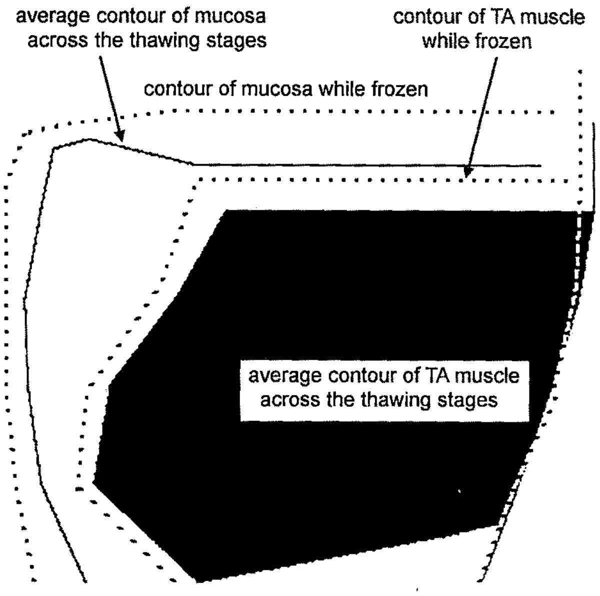
average contour of mucosa across the thawing stages
contour of TA muscle while frozen
contour of mucosa while frozen
average contour of TA muscle across the thawing stages

Fig 4. Reconstruction of vocal fold contour across frozen and thawing stages.

activity tend to bulge out medially along the inferior vocal fold border, creating a deeper and thicker vibrating structure with a more rectangular glottal shape.[22] These two vocal fold configurations are believed to be associated with the falsetto voice (vocal register) and the chest voice (or modal register), respectively.[22] Consequently, one major limitation of the present study was that geometric data were obtained from excised canine larynges, which obviously did not have any TA muscle activity. Nonetheless, the present data are representative of the resting vocal fold geometry, which are useful for establishing the baseline geometric configuration of laryngeal biomechanical models. Further studies are needed to quantify dynamic changes of vocal fold geometry as a function of TA muscle activity.

CONCLUSION

Data on vocal fold geometry are important information for the biomechanical modeling of voice production. In this study, we operationally defined the depth and the thickness of the vocal fold with respect to the laryngeal framework and the TA muscle, so as to efficiently represent the vocal fold geometry in laryngeal biomechanical models. In order to minimize the significant tissue deformation typically associated with histologic techniques, we used the alternative method of quick-freezing for preparing midmembranous coronal sections of canine vocal folds. Measurements of the vocal fold dimensions were made on quick-frozen and thawing canine tissues with digital photography. The results are useful for defining the vocal fold boundaries with respect to the laryngeal framework in biomechanical models of the larynx, especially for continuum-mechanical models (eg, finite-element models[21]) that are based on the physiological properties (active contractile properties) of the canine laryngeal muscles[1-3] (also Tayama et al, unpublished data).

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LONG-TERM RESULTS OF THE STERNOHYOID MYOCUTANEOUS ROTARY DOOR FLAP FOR LARYNGOTRACHEAL RECONSTRUCTION

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In the past 8 years, 45 patients with laryngotracheal stenosis have undergone reconstruction with the sternohyoid myocutaneous rotary door flap (RDF) technique at Tang Du Teaching Hospital in Xi'an, China. All patients were tracheostomy-dependent before reconstruction. Thirty-six patients had previously undergone 2 to 7 surgical procedures. Forty-one of the 45 patients (91%) were successfully decannulated. Decannulation failed in the other 4 because of RDF prolapse into the tracheal lumen or because of hypertrophic scar in the operative region. The follow-up period ranged from 1 to 8 years. Forty of the 41 patients had a stable airway and effective voice, and 1 patient had restenosis 3 years after decannulation. The results demonstrate that the RDF technique is a relatively simple and effective way to treat patients with laryngotracheal stenosis.

KEY WORDS — laryngotracheal reconstruction, laryngotracheal stenosis, myocutaneous flaps.

INTRODUCTION

Over many years, a variety of reconstructive procedures have been designed for the treatment of laryngotracheal stenosis. Augmentation of the circumference of the airway with an open surgical technique is one of the most successful methods of laryngotracheal reconstruction. Autogenous cartilage or epiglottis or hyoid bone grafts have been used to increase the intraluminal diameter of the airway with various degrees of success,[1-3] but no single technique can be universally applied. Eliachar et al[4] first reported a series of successful cases of laryngotracheal reconstruction using a sternohyoid myocutaneous rotary door flap (RDF) technique. The RDF is a regional bipedicled flap with a good blood supply. The ability of the flap to increase the intraluminal diameter of the stenotic area depends on the dynamic tension of the rotation of the bipedicled myocutaneous flap. It does not need skeletal support.[5] In this article we present the long-term results of a series of 45 patients with laryngotracheal stenosis who underwent laryngotracheal reconstruction with the RDF technique in the past 8 years.

MATERIALS AND METHODS

Forty-five patients with laryngotracheal stenosis underwent laryngotracheal reconstruction by the RDF technique at Tang Du Teaching Hospital in Xi'an, China, from 1990 to 1998. Their ages ranged from 3 to 71 years (mean age, 30 years); 32 of the 45 patients were male. The cause, location, and severity of stenosis are shown in the Table.[6] The length of the stenosis varied from 1 to 6 cm, with a mean of 3.5 cm. All patients were tracheostomy-dependent. Thirty-five patients had previously undergone between 2 and 7 surgical procedures (average, 3.4), including dilation, hyoid bone transposition, and laser resection.

With the patient in the supine position, general anesthesia was administered via the previously placed tracheostomy. The skin was prepared in a sterile manner. Laryngotracheal reconstruction was performed by the RDF method as described by Eliachar et al.[4] A vertical midline incision was made between the

PATIENT DATA

Parameter	*No. of Patients*
Cause of stenosis	
Trauma	31
Prolonged intubation	5
Partial laryngectomy	3
Tracheotomy	3
Respiratory scleroma	1
Laryngotracheal amyloid	1
Unknown	1
Location of stenosis	
Glottis	4
Glottis and subglottis	16
Subglottis	10
Subglottis and trachea	11
Trachea	4
Stenosis grade[6]	
1	0
2	8
3	27
4	10

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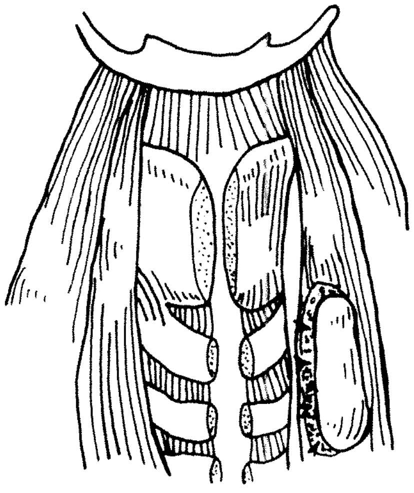

Fig 1. Rotary door flap has been dissected and prepared for rotation into stenosis site.

strap muscles to expose the thyroid cartilage, cricoid arch, and trachea. Care was taken to prevent shearing of the sternohyoid muscle and the corresponding overlying skin. The cricoid arch and trachea were divided at the midline; the superior and inferior extensions were based on the extent of the stenosis. Scar tissue was removed submucosally by making a Z-shaped incision in the posterior wall of the airway. The flaps of the "Z" were then reversed and sutured in place after the scar had been resected.

A vertical paramedian RDF was outlined over the sternohyoid muscle, situated so that it would lie at the proper level and be of sufficient length and width to repair the known laryngotracheal stenosis. The subcutaneous and dermal tissues were tacked down to the fascia and the muscle with interrupted absorbable sutures to prevent shearing of the muscle and skin (Fig 1). The sternohyoid muscle was elevated from both its sternum attachment and its hyoid bone insertion. The blood vessels were carefully dissected and preserved. Release of tension permitted 180° rotation of the flap to cover the defect in the airway. The RDF was turned on its longitudinal axis into position (Fig 2). The flap was placed skin surface–down over the opening in the airway and sutured to the cartilage along the margins of the defect. The wound was irrigated. The adjacent neck skin was advanced, approximated, and closed. The wound was drained with a suction drain. The enlarged lumen was stabilized with an oversized silicone T-tube.

There were 20 patients with concomitant bilateral vocal cord cicatricial stenosis, paralysis, or severe posterior glottic cicatricial shrinkage. Arytenoidectomy and lateralization of one vocal cord, or a posterior cricoid split with grafts, was carried out in these patients.

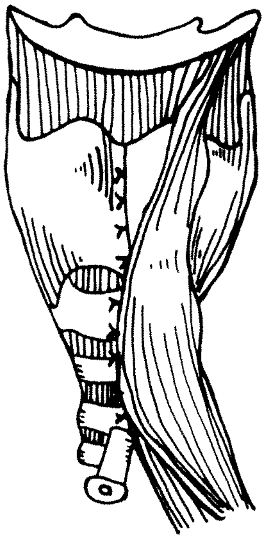

Fig 2. Rotary door flap is rotated into position on its longitudinal axis, and skin flap is sutured to edges of tracheal defect over T-tube.

A nasogastric tube was introduced for postoperative feeding for 5 to 6 days. Prophylactic antibiotics were used. The T-tube was removed from 1 to 12 months after operation (average, 6 months). After removal of the T-tube, a small tracheostomy tube was placed and corked. If the patient had good airway function with the tube blocked for 7 to 10 days and the endoscopic examination showed a stable airway, decannulation was performed.

RESULTS

Forty-one of the 45 patients were successfully decannulated. Two patients had RDF prolapse into the tracheal lumen that required another open procedure for reconstruction, and decannulation failed in the other 2 patients because of hypertrophic scar in the operative region. All decannulated patients had a stable airway on endoscopy. The graft appeared pinkish, with no crust or desquamation. These patients had a normal exercise tolerance and a functional voice. The patients with glottic stenosis had a weak voice.

The length of follow-up was from 1 to 8 years in the 41 patients. Forty of the 41 patients had a stable airway and an effective voice. One patient, in whom the cause of stenosis was undefined, had restenosis 3 years after decannulation. He remains tracheostomy-dependent.

There were 3 complications among these patients. Two patients who underwent RDF and a concomitant posterior cricoid split procedure developed wound bleeding on the second or third day after operation. Emergency laryngofissure was performed to electrocoagulate the bleeding. Three patients developed a wound infection that required use of a T-tube for 12 months. Three patients had excessive granulation tissue around the myocutaneous suture line within the airway. The granulation tissue was removed endoscopically.

DISCUSSION

The ideal tissue graft for augmenting the circumference of the laryngotracheal lumen should have an epithelialized surface to prevent narrowing of the lumen, a framework to maintain the airway, and a good blood supply. The RDF technique fulfills these requirements. The sternohyoid myocutaneous RDF is a bipedicled flap with an abundant blood supply that derives from the superior and inferior thyroid arteries. The epithelialized surface is the skin. The airway support is provided by the dynamic tension of the rotated myocutaneous flap. The disadvantage of this method is the skin involuting into the airway, which may lead to crusting and desquamation.

In this study, 41 of 45 patients (91%) were decannulated. Follow-up for 1 to 8 years in the 41 patients showed that restenosis developed in only 1 patient. All other patients had a stable airway and resumed their usual daily activities. In our experience, this technique may be contraindicated in patients with abundant subcutaneous fat or with a tendency to scar formation. Careful hemostasis is important in patients who undergo a concomitant posterior cricoid split. Prophylactic antibiotics and local wound care are important for preventing complications.

In conclusion, the sternohyoid myocutaneous RDF is a relatively simple method and has good long-term results for laryngotracheal reconstruction in selected cases. The complications are few and minor. This method may be contraindicated in patients with a tendency to scar formation or subcutaneous lipotrophy.

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LYMPHATIC METASTASIS TO THE SUPRARETROSPINAL RECESS IN LARYNGEAL SQUAMOUS CELL CARCINOMA

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In order to evaluate lymphatic metastasis to the supraretrospinal recess (SRSR) in laryngeal squamous cell carcinoma (SCC), we separately dissected SRSR lymph nodes and submitted them to pathological examination. Fifty-three lateral neck dissections (LNDs), 2 radical neck dissections (RNDs), and 19 modified RNDs were performed in 49 previously untreated patients with laryngeal SCC. The nodal status of the patients was N0 in 29 patients, N1 in 17, and N2 in 3. The neck was pathologically positive in both RNDs (100%), in 7 of 19 modified RNDs (37%), and in 7 of 53 LNDs (13%). No SRSR lymph nodes were positive in any of the dissection materials. No metastasis was found in the SRSR lymph nodes in the N0 necks treated with LND, and none was found even in N1 and N2 necks treated with RND or modified RND. We conclude that the SRSR may be left undissected during treatment of an N0 neck with LND so that accessory nerve dysfunction can be minimized and operative time can be saved.

KEY WORDS — carcinoma, larynx, neck metastasis, selective neck dissection.

INTRODUCTION

Neck metastasis from primary head and neck squamous cell carcinoma (SCC) has prognostic importance; in cases with regional metastasis, the cure rate decreases.[1-3] Radical neck dissection (RND) has been a well-accepted surgical procedure for the treatment of neck metastasis since the beginning of the 20th century. It results in cosmetic and functional deficits, and more conservative therapeutic approaches that have better functional results have been introduced into the surgical literature.[4,5] Results of studies on the nodal spread pattern of different primary sites in the upper aerodigestive tract[1,5-7] motivated surgeons to perform limited neck dissection and gave rise to the concept of selective lymph node dissection.[8] In the neck, levels II, III, and IV are the most involved lymphatic groups in laryngeal carcinoma,[1,7,9,10] and removal of these lymph node levels is called lateral selective neck dissection (LND).[8] The primary goal of this procedure is to stage the neck and to treat occult metastasis.

The upper part of the area dissected in LND (level II) has a close relationship with the accessory nerve (AN); this nerve crosses level II obliquely in a superoinferior and mediolateral direction. This course of the AN divides level II into 2 parts, the posterosuperolateral part of which was named level IIb by Suen and Goepfert[11] and was named the submuscular recess by Calearo and Teatini[12] and Talmi et al.[13] Lymph nodes dissected from this recess were termed supraspinal accessory lymph nodes (SSALNs) by Kraus et al.[14] This recess is bounded anteromedially by the AN itself, superiorly by the posterior belly of the digastric muscle, and posterolaterally by the sternocleidomastoid muscle. The levator scapula and splenius capitis muscles compose the bed of this anatomic area. Because this area is superior to and behind the AN, we believe the term supraretrospinal recess (SRSR) is more definitive.

Metastasis to this group of lymph nodes has been the subject of only a few studies, and those studies dealt mainly with primaries of the oral cavity, the oropharynx,[14] or multiple sites of the head and neck.[13] Because of the paucity of studies dealing with lymphatic metastasis to the SRSR in laryngeal carcinoma, a prospective study was planned to reveal lymph node metastasis to this recess in laryngeal SCC.

MATERIALS AND METHODS

Our study group consisted of 49 previously untreated patients in whom diagnosis of laryngeal SCC was made between April 1998 and April 1999. Each patient was staged according to the American Joint Committee on Cancer TNM classification system.[15] Neck dissection was performed or supervised by the senior author (A.K.).

The SRSR specimens were removed from the surgical area during dissection of the upper jugular nodes.[14] They were examined while fresh, together with the rest of the neck dissection material, and were separately submitted for permanent pathological section.

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TABLE 1. T AND N STATUS OF PATIENTS

	N0	*N1*	*N2*
T1	3		
T2	16	4	
T3	10	10	1
T4		3	2

RESULTS

All patients were male; their ages ranged from 38 to 74 years, with a mean of 56 years. The tumors were glottic in 21 patients, supraglottic in 16, and transglottic in 12. The primary tumor stages included 3 T1, 20 T2, 21 T3, and 5 T4 laryngeal SCCs (Table 1). Seventy-four neck dissections were performed at the same time as the resection of primaries. The nodal statuses of the patients were as follows: 29 N0, 17 N1, and 3 N2. In patients with N2 necks, 2 RNDs and 1 modified RND (MRND) were performed ipsilaterally, and 1 MRND and 2 LNDs were performed contralaterally. All N1 necks were treated with MRND on the side of the primary tumor and with LND on the contralateral side. Thirty-four LNDs were performed in 29 N0 patients (5 patients underwent LND on both sides; Table 2).

We dissected 5 to 50 lymph nodes from patients who underwent LNDs (mean, 28.7), 23 to 102 nodes from patients who underwent RNDs and MRNDs (mean, 51.5), and 1 to 10 nodes from the SRSR (mean, 3.9; Table 3).

Positive lymph nodes were found in 2 patients who had undergone RND: at levels II and III in one and at levels III and IV in the other. In patients who underwent MRND, lymph nodes were positive at levels II and III in 1 patient, at levels III and IV in 1 patient, at only level II in 3 patients (1 on the contralateral side), and at only level III in 2 patients. In patients treated with LND, positive metastatic nodes were found at level II in 6 patients and at level III in 1 patient (Table 4).

Neck levels I and V were free of nodal metastasis for the RNDs and MRNDs; all metastatic lymph nodes were at levels II, III, and IV of the neck. Separately dissected SRSR lymph nodes were also free of disease.

TABLE 2. PERFORMED DISSECTIONS ACCORDING TO N STATUS

N Status	*No. of Patients*	*Ipsilateral Dissection*	*Contralateral Dissection*
N2	3	2 RND, 1 MRND	1 MRND, 2 LND
N1	17	17 MRND	17 LND
N0	29	29 LND	5 LND
Total	49	49	25

RND — radical neck dissection, MRND — modified radical neck dissection, LND — lateral neck dissection.

TABLE 3. NUMBERS OF LYMPH NODES HARVESTED FROM VARIOUS NECK LEVELS

	Harvested Lymph Nodes	
Dissection Levels	*Range*	*Mean*
I to V (RND, MRND)	23-102	51.5
II to IV (LND)	5-50	28.7
IIb (SRSR)	1-10	3.9

SRSR — supraretrospinal recess.

DISCUSSION

The efficacy of selective neck dissection in the treatment of upper aerodigestive tract SCC has been highlighted in several studies.[16-18] There are 3 patterns of neck recurrence following selective neck dissection: 1) within the dissection field, 2) outside the dissection field, and 3) in the contralateral, undissected side of the neck.[16]

Spiro et al[17] performed 85 selective neck dissections in 66 N0 and N1 patients with SCC of the larynx and pharynx. Six patients (7%) had recurrence: 5 outside the operative field and 1 within the limits of the previous jugular neck dissection. They concluded that selective jugular neck dissection for N0 laryngeal and pharyngeal SCC was a reliable method of treatment, and that for N1 disease, limited lymphadenectomy with aggressive radiotherapy might yield comparable results in carefully selected patients. In a retrospective study by Byers et al,[16] 517 selective neck dissections were performed in 363 patients with upper aerodigestive tract SCC. Of these procedures, 192 were LNDs and were performed in patients with a primary tumor in the larynx and hypopharynx. There were 25 recurrences in the neck (13%), and 12 of these recurrences (6%) were in the previously dissected field. In their article, Byers et al mainly focused on recurrences in the supraomohyoid dissection group and did not mention recurrences in the LND group. Pellitteri et al[18] saw only 1 recurrence in 25 patients (4%) who underwent LND for laryngeal and hypopharyngeal SCC and were followed up for more than 2 years. The recurrence was in a previously dissected field at level III in a patient who had had multiple pathologically positive lymph nodes. All N0 and N1 necks were free of disease in the follow-up period.

Level II makes up the superior part of the area

TABLE 4. POSITIVE LYMPH NODES AND NECK LEVELS

Dissection Type	*Extent of Involvement (%)*	*No. of Patients (level)*
RND	100% (2/2)	1 (II, III), 1 (III, IV)
MRND	37% (7/19)	3 (II), 2 (III), 1 (III, IV), 1 (II, III)
LND	13% (7/53)	6 (II), 1 (III)

dissected in LND and is in close proximity to level V; the AN traverses this level obliquely. The spinal accessory lymph nodes are adjacent to the upper jugular nodes, and there is no boundary between the upper jugular and AN lymph nodes in the area in which the SRSR lymph nodes are also located. In RND and in MRND with a widely exposed surgical field, in which the AN is spared, dissection of the lymph nodes in the SRSR does not cause any problems. In LND, the posterior boundary of the dissection is the posterior border of the sternocleidomastoid muscle,[8] and as part of the surgery, the lymph nodes in the SRSR are included in the dissection material.

Schuller et al[19] noted the frequency of accessory lymph node metastasis and divided these nodes into superior and inferior, the reference point being the entrance of the AN into the sternocleidomastoid muscle. They found histologically documented metastatic disease in 28 of 50 patients, and the superior accessory lymph nodes were positive in 20 of these 28 patients (71.4%). In their study, a node was determined as a spinal accessory node when it was adjacent to the nerve, but the exact location of the nodes (whether they belonged to level IIa or level IIb) was not mentioned in this study.

Byers[5] proposed that dissection of upper posterior cervical lymph nodes is the most important and difficult part of any type of MRND. He recommended removal of the sternocleidomastoid muscle and the internal jugular vein to prevent neck recurrence if a positive upper posterior cervical node is discovered at the time of dissection. Removal of the internal jugular vein was also recommended by Traynor et al[20] for reliable dissection of the jugular lymphatic chain.

Specific study of SRSR lymph node metastasis was first done by Kraus et al[14] in 1996. They designed a study to identify the incidence of SSALN metastases in supraomohyoid neck dissection for oral cavity and oropharyngeal primaries. They found only 1 SSALN metastasis in a patient with T3N0 carcinoma of the tonsil out of 47 patients (2%), and this patient also had lymph node metastasis at level II. There was no isolated SSALN metastasis, and Kraus et al concluded that there is a low incidence of SSALN metastasis in patients with T+N0 SCC of the oral cavity and oropharynx.

Another study was carried out by Talmi et al[13] for various head and neck primaries. They performed 102 neck dissections (80 selective, 22 RND or MRND) in 71 patients with SCC of the head and neck. Submuscular recess (termed by us SRSR) lymph nodes were positive in 4 patients. The primary sites were the alveolar ridge, the anterior part of the tongue, and the nasopharynx; in the other patient, the primary site was unknown. All necks in the study were staged before surgery as N2 or greater. Fourteen of the 71 patients constituted the laryngeal cancer subgroup (17 neck dissections), and the submuscular recess (SRSR) was negative in all of those patients.

Our study group consisted of laryngeal SCC patients only. The majority of our cases were N0 and N1 necks (29 and 17 patients, respectively); there were 3 N2 necks. In all of our cases, the SRSR lymph nodes were pathologically negative. It can be stated that laryngeal SCCs do not metastasize to the SRSR, especially in N0 and N1 necks. Because the number of N2 necks was limited in our study, it is difficult to extrapolate our results to N2 necks.

The number of lymph nodes dissected from the SRSR varied between 2 and 9 in the study done by Talmi et al[13] and between 0 and 13 in the study by Kraus et al,[14] with means of 4.2 and 4, respectively. In our study, the number of dissected lymph nodes in the SRSR was between 1 and 10 (mean, 3.9). Although this area has a limited number of lymph nodes, attempts to dissect these lymph nodes, especially in selective neck dissections with retraction of the sternocleidomastoid muscle, prolong the duration of the operation and may adversely affect the function of the AN. Sparing the anatomic integrity of the AN does not always result in an optimal functional outcome; neurapraxia can be caused by traction applied to the AN during dissection of the SRSR, and this effect is most prominent in the early postoperative period.[21-23] It is probable that leaving the SRSR undissected will result in a minimal deteriorative effect on the AN, as well as decreased operative time.

In conclusion, in our study, no metastasis was found in the SRSR lymph nodes in the N0 necks treated with LND, and none was found even in N1 and N2 necks treated with RND and MRND. Therefore, the SRSR lymph nodes may be left undissected during treatment of an N0 neck with LND so that AN dysfunction can be minimized and operative time can be saved.

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IMAGING CASE STUDY OF THE MONTH

MASSIVE BILATERAL INFERIOR CONCHA BULLOSA

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MERSİN, TURKEY

INTRODUCTION

Pneumatization of the middle turbinate and, less commonly, of the superior turbinate is known as concha bullosa.[1] Inferior concha bullosa (ICB) is an air-containing structure in the body of the inferior turbinate that may communicate with the maxillary sinus. Although concha bullosa of the middle turbinate is very common, ICB is extremely rare.[2] In the English-language literature, there are 3 reported unilateral ICBs and 1 bilateral ICB.[2-4] Herein, we present a case of bilateral ICB associated with unilateral concha bullosa of the middle turbinate, septal pneumatization, and unilateral overpneumatized ethmoidal bulla.

CASE REPORT

A 35-year-old woman presented with the complaints of nasal obstruction and facial headache. She had had the symptoms for 3 years, and there was no history of either trauma or nasal allergy. Physical and nasal endoscopic examination revealed left middle turbinate and bilateral inferior turbinate hypertrophy and right septal deviation. The patient was otherwise healthy. Coronal computed tomography (CT) performed with 3-mm collimation and interval showed bilateral inferior and left middle concha bullosa, left overpneumatized ethmoid bulla, posterior septal pneumatization, and right septal deviation. The aerated cavity within the turbinates did not communicate with one of the paranasal sinuses, but rather, with the nasal cavity through a wide opening (see Figure). Septoplasty, lateral excision of the middle concha bullosa, and bilateral inferior concha outfracture were undertaken, and Merocell was used for nasal packing. The postoperative course was uneventful. Follow-up nasal endoscopic examinations revealed that the nasal airway was adequate. One year after the operation, the patient was free of nasal complaints.

DISCUSSION

The inferior turbinate is a scroll of bone that extends from the nares anteriorly to the choanae posteriorly. It overhangs and delineates the inferior meatus, significant only for the nasolacrimal duct orifice that opens at the anterior end of the meatus.[5] Variations of the nasal cavity are very important for otolaryngologists, especially in functional endoscopic sinus surgery. Although the characteristics of middle concha bullosa have been adequately evaluated, the mechanism of the formation of ICB is not fully explained in the literature.[4,6] Two mechanisms reported for ICB formation are 1) the ossification of the chondral framework of the inferior concha to a double lamella in fetal life and the misinvagination of the epithelium into the mentioned double lamella suggested by Dogru et al[3] and 2) air cell formation associated intimately with inferior turbinate attachment formed by maxillary sinus disease, explained by Namon.[7]

Since conventional radiology does not permit a detailed study of the nasal cavity and the paranasal sinuses, it has now largely been replaced by CT. Inferior turbinate enlargement generally presents with nasal obstruction, rhinorrhea, or postnasal drainage. No single procedure can be suitable for every case of turbinate hypertrophy. A long list of procedures has been suggested to deal with hypertrophy of the inferior turbinate, including submucosal injection of sclerosants or corticosteroids, cryoturbinectomy, and resection of the turbinate.[2,8]

No surgical treatment method has been reported, other than the procedure performed by Dogru et al,[3] which is the excision of the lateral aspect of the inferior turbinate in an anterior-posterior direction. We performed lateral wall resection of the middle concha bullosa with outfracture of both inferior turbinates to gain access to nasal airflow.

From the Departments of Otorhinolaryngology (Özcan, Görür) and Radiology (Duce), Mersin University, School of Medicine, Mersin, Turkey.
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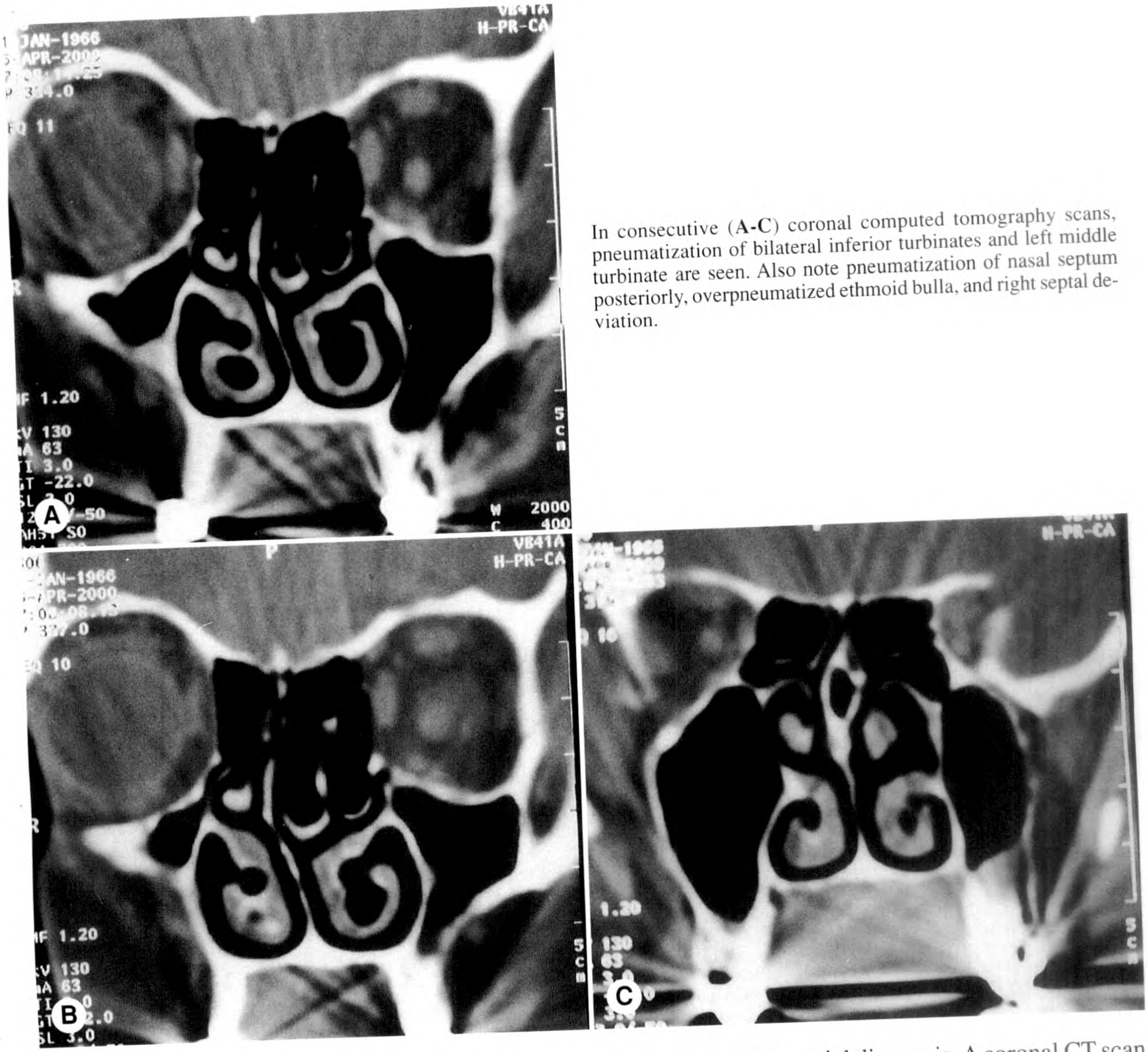

In consecutive (**A-C**) coronal computed tomography scans, pneumatization of bilateral inferior turbinates and left middle turbinate are seen. Also note pneumatization of nasal septum posteriorly, overpneumatized ethmoid bulla, and right septal deviation.

In the first postoperative year, the patient's symptom-free history confirmed the validity of our preoperative evaluation and the sufficiency of our surgical procedure. In cases of inferior concha hypertrophy, ICB variation should be taken into consideration in the differential diagnosis. A coronal CT scan is of great value in the diagnosis, not just for investigation prior to nasal surgery, but also to alert the surgeon to possible anatomic variations of the inferior concha.

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BOOK REVIEWS

Hair Cells Micromechanics and Hearing

Charles I. Berlin and Richard P. Bobbin, editors. Hard cover, illustrated, indexed, 162 pages, 2001. Singular Thompson Learning, San Diego, Calif, $71.95.

Purpose: The volume represents the integration of the latest information and thinking on hair cells and their auditory function. This is the sixth book in the Kresge-Mirmelstein Award Series, which honors an outstanding auditory scientist each year.

Contents: The book is a collection of 7 manuscripts from the proceedings of a symposium honoring David Corey, PhD, the 1999 Kresge-Mirmelstein Award recipient and author of the first chapter, for his discoveries on hair cell micromechanics. The remaining 6 chapters were contributed by other investigators invited to present papers related to Dr Corey's work. The first chapter reviews the two general mechanisms proposed for adaptation. The following sections present material on "self-repair" of hair cells, fast adaptation as a mechanical tuning mechanism, sea anemone versus vertebrate hair cells, gene regulation of hair cell differentiation, the role of metabotropic glutamate receptors in transmitter release, and the roles of adenosine triphosphate (ATP) and ATP receptors in modulation of cochlear mechanics and the control of ion flow.

Attributes: The collection of manuscripts brings to the forefront the newest and most exciting studies regarding hair cells and their function in the auditory system. Dr Corey's contribution is a particularly useful review of transduction and adaptation, making clearer both hair cell physiology and function. Other data presented, such as that of Dr Gene Watson regarding self-repair of hair cells, or that of Dr Allen Ryan showing that the gene regulatory factor Brn-3.1 is required for differentiation of hair cells from their precursors, may have great impact on future discoveries in human auditory development and function and the treatment of auditory disorders. The book is a valuable reference for the clinical researcher or the accomplished graduate student interested in hair cell micromechanics.

Limitations: The depth of assumed knowledge of cellular and molecular biology and biochemistry may be too great. This could be of particular importance, as the text is likely to be included in the curricula for graduate programs in audiology and otolaryngology.

Applicability: The book is intended for clinical researchers in audiology and otolaryngology. It may also prove useful for instruction of graduate students in those disciplines.

Ann Elizabeth Jacobson, AuD Candidate
Dennis G. Pappas, MD
Birmingham, Alabama

Hearing: Its Physiology and Pathophysiology

Aage R. Møller. Hard cover, illustrated, indexed, 515 pages, 2000. Academic Press, San Diego, Calif, $79.95.

Purpose: To provide a thorough description of coding of complex sounds in the auditory system and a discussion of the analysis that occurs in the ear and the auditory nervous system. Also, to discuss the effect of disorders of the cochlea and the auditory nervous system on processing of complex sounds in a way that is applicable to providing up-to-date health care.

Contents: Uniquely, this book is made up of 4 sections that include the ear, the auditory nervous system, acoustic reflexes, disorders of the auditory system, and their pathophysiology. But within each main division, there are many subdivisions that include varied topics such as anatomy, physiology, and pathology that are specific to the main division.

Attributes and Limitations: Scientific study of the theory of hearing began with Hermann Helmholtz's theories of mechanically tuned resonant features located along the basilar membrane (1860s). The second thrust was Georg von Békésy's fea-tures of spectral analysis in the cochlea resulting in a traveling wave motion along the basilar membrane. The third, and most recent, development in the theory of hearing involves the trans-mission of energy into motion of the basement membrane, pre-paring sounds for temporal coding. Such a process involves the "muscular" action of the outer hair cells that increase the vibra-tion amplitude of the basement membrane, while the friction on the motion of the basilar membrane is reduced.

Many scientists have participated in the work on inner and outer hair cell sensory transduction. Dr Møller simply and clearly presents this material and shows how the auditory system works. There are no complicating factors such as physics. The book describes the pathophysiology of disorders that affect the middle ear, the cochlea, the auditory nerve, and even the higher auditory nerve structures. Disorders such as acoustic tumors, tinnitus, and lesions are discussed and represent a synthesis of the latest information and thinking on auditory function.

Applicability: Clinicians, clinical researchers, and basic scientists will all benefit from this book.

Dennis G. Pappas, MD
Birmingham, Alabama

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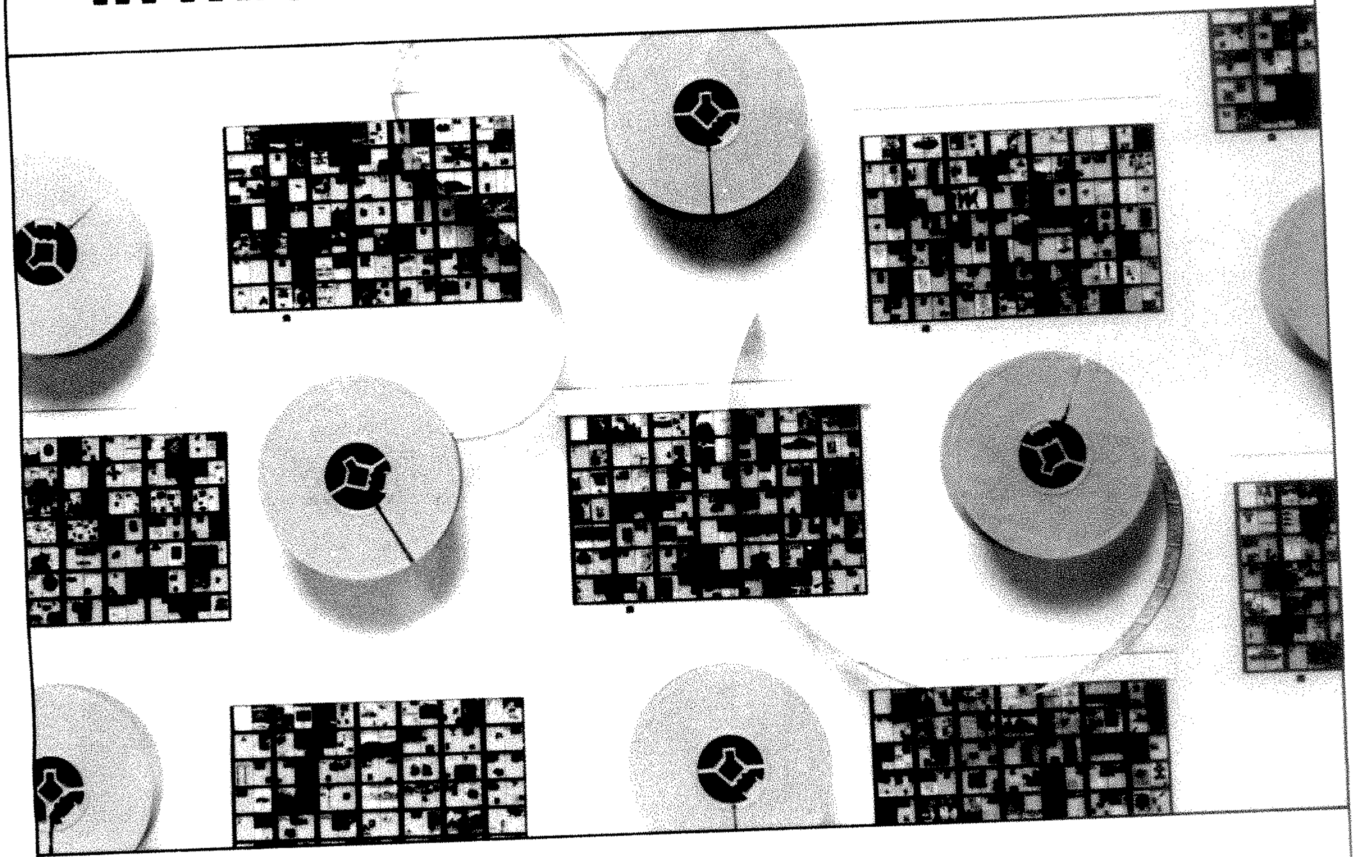

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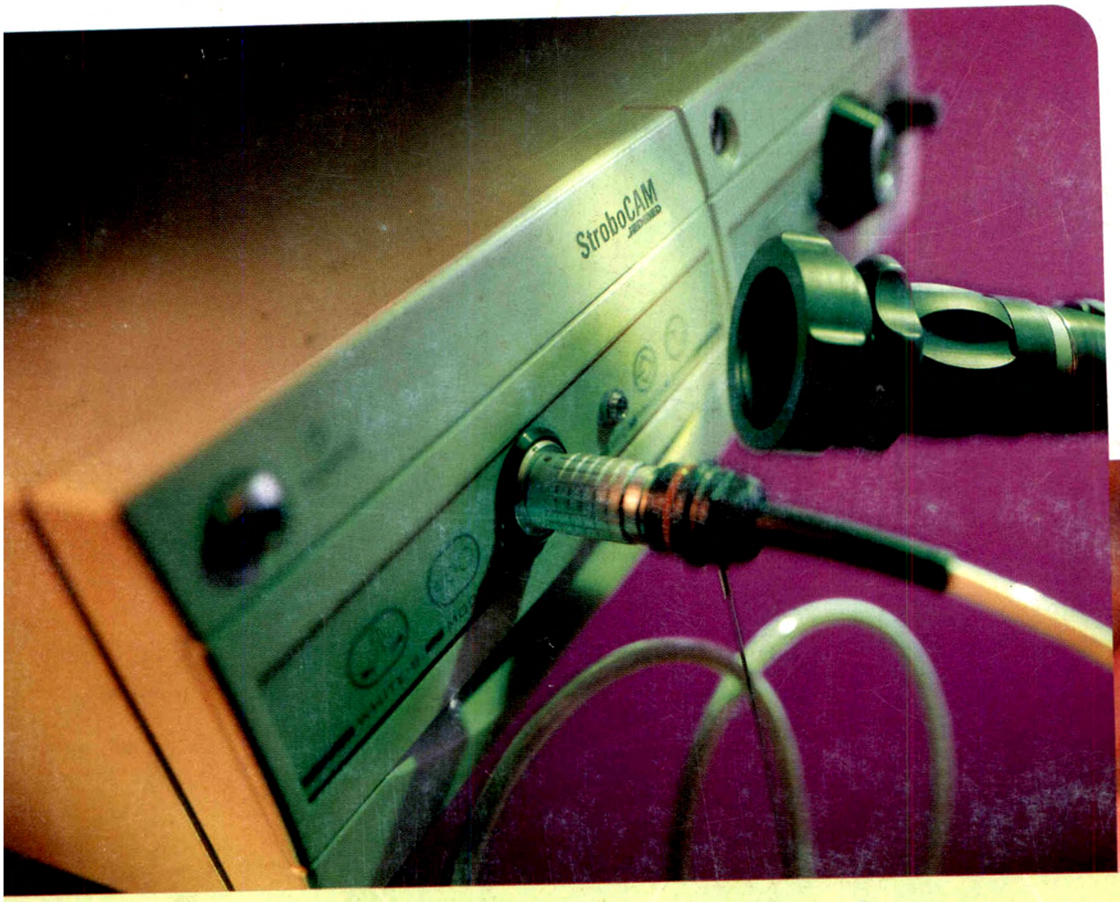

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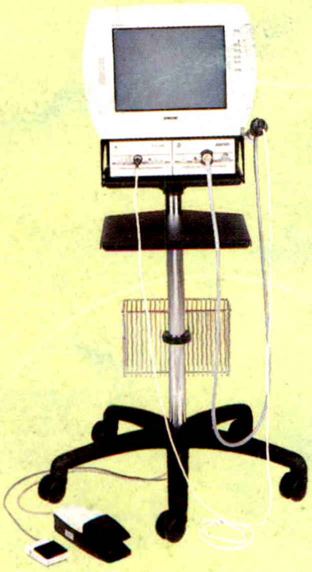

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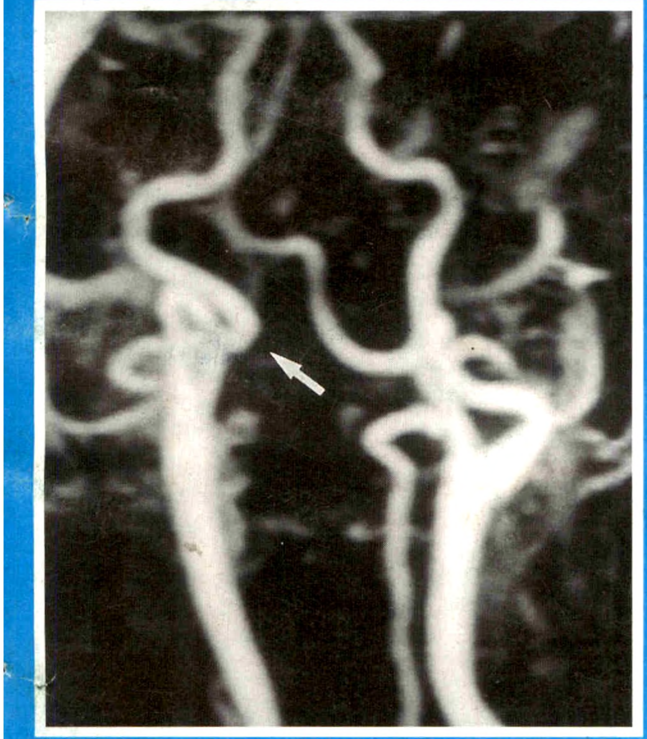

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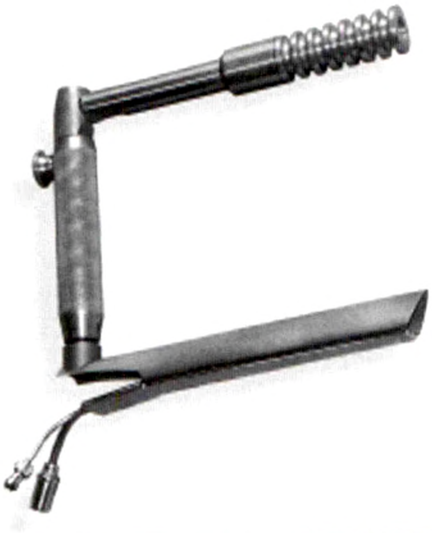

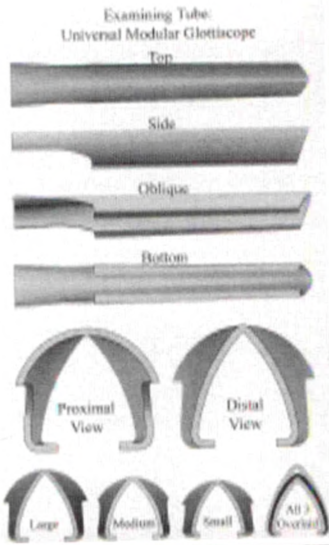

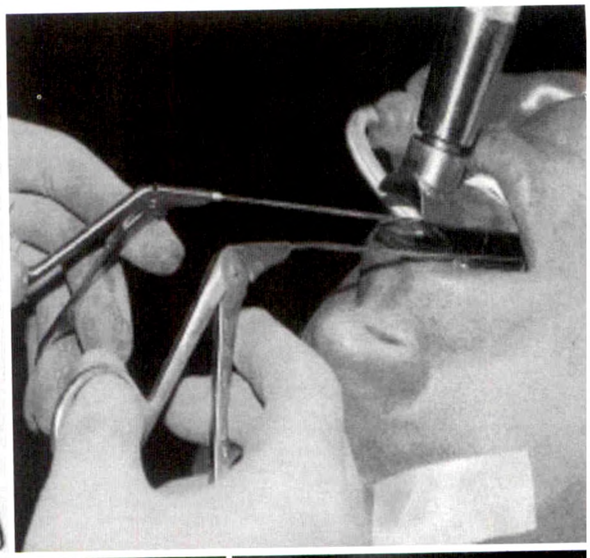

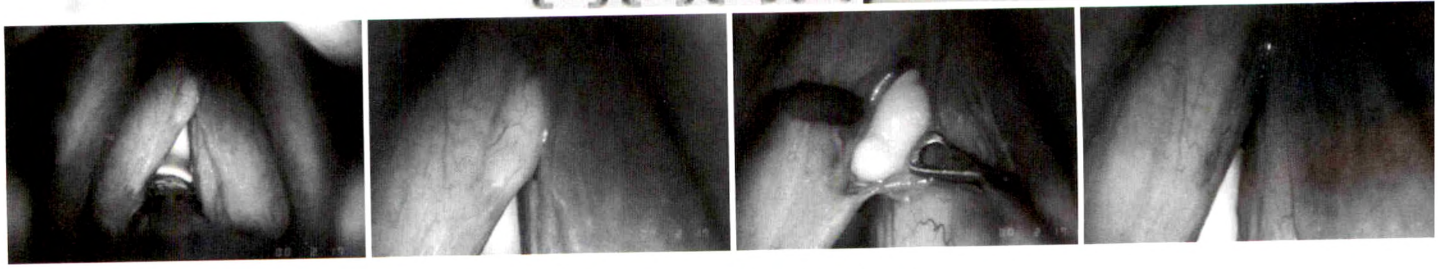

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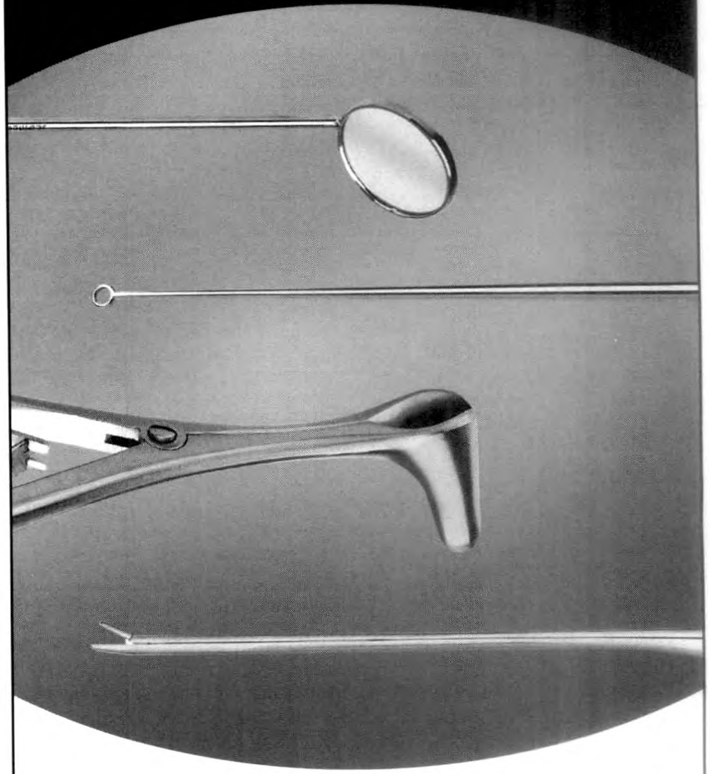

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THE THREE FACES OF VESTIBULAR GANGLIONITIS

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MOBILE, ALABAMA

We present temporal bone and clinical evidence that common syndromes of recurrent vertigo are caused by a viral infection of the vestibular ganglion. In the present series, histopathologic and radiologic changes in the vestibular ganglion and meatal ganglion were consistent with a viral inflammation of ganglion cells in cases of Meniere's disease, benign paroxysmal positional vertigo, and vestibular neuronitis. Clinical observations of multiple neuropathies involving cranial nerves V, VII, and VIII on the same side in patients with recurrent vertigo are best explained by a cranial polyganglionitis caused by a neurotrophic virus, which is reactivated by a stressful event later in life. The reactivation of the latent virus may manifest as one of the above vertigo syndromes, depending on the part of the vestibular ganglion that is inflamed, the type and strain of the virus, and host resistance.

KEY WORDS — benign paroxysmal positional vertigo, Meniere's disease, vestibular ganglionitis, vestibular neuronitis.

Intermittent vertigo represents one of the most disabling symptoms encountered in otologic practice. The recurrent nature of this disability implies a reversible alteration in vestibular nerve physiological function caused by changes in the neuron or its environment. Three of the most common clinical syndromes that manifest as recurrent vertigo are benign paroxysmal positional vertigo (BPV), Meniere's disease (MD), and vestibular neuronitis (VN).

Several features of MD and BPV are not accounted for by currently held concepts of pathophysiology. In MD, ruptures of a distended membranous labyrinth with release into the perilymphatic space of high-potassium endolymph, which is toxic to neuronal activity, are thought to be responsible for repeated vertiginous episodes and a fluctuating sensorineural hearing loss.[1-3] The concept of endolymphatic hydrops (EH) as the underlying pathophysiological mechanism in MD[4-6] receives support from an animal model of EH following endolymphatic duct obstruction.[7,8]

Although EH is readily produced in chinchillas, guinea pigs, and rabbits, comparable EH is not achieved when the endolymphatic sac is obstructed in higher mammals (ie, monkeys and cats).[9,10] Furthermore, the absence of vestibular asymmetry (vertigo) in animal models of EH and the lack of perilymphatic space fibrosis in the animals' temporal bones (TBs) conflict with the clinical and histopathologic features of MD. Medical and surgical treatments designed to reduce fluid in the labyrinth have failed to achieve relief from vertigo comparable to that attained after vestibular ablation. These observations raise doubt about endolymphatic sac dysfunction as the responsible pathological correlate in MD. On the other hand, degenerative and inflammatory changes in surgically excised vestibular nerve ganglia of MD patients have been reported with increasing frequency.[11-13]

The pathophysiology of BPV has been based on a change in the motion mechanics of the labyrinth sense organs. Deposits of high specific gravity (possibly otoliths) have been observed fixed to the cupula or free-floating in the membranous limb of the posterior semicircular canal. These particles are thought to convert the crista into a receptor that responds to gravitational forces when provoked by the changes in head position. However, this mechanical concept does not account for the limited duration of nystagmus in the maintained provocative position, its fatigability on repeated provocation, or the prolonged periods of remission between episodes of position-induced vertigo.[14,15] Furthermore, an absence of gravity-sensitive posterior semicircular canal cupular or free-floating deposits and the presence of focal neural degeneration in the inferior vestibular division have been reported in the TBs of patients with clinical features of BPV.[16]

Inconsistencies between the histopathologic and clinical features of these balance disorders call into question the validity of pathophysiological mechanisms based solely on end organ alteration. On the other hand, the lesion responsible for VN has long been regarded as a degeneration of vestibular ganglion cells, probably caused by a viral agent.[17,18] Epidemiological,[19-21] clinical,[22-24] and histopathologic[25-27] evidence supports a viral cause for the recurrent episodes of vertigo without an associated hearing loss.

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Furthermore, viral antigen can be detected in vestibular ganglion (VG) cells when a neurotropic (NT) strain of mumps virus is inoculated into the labyrinth of hamsters.[18] Although a significant decrease in vestibular sensitivity is a major criterion for establishing the diagnosis of VN,[22] it is not uncommon in acute VN to observe reversal of a decreased vestibular response[28,29] and focal enhancement in the internal auditory canal on contrast magnetic resonance imaging (MRI) after resolution of clinical symptoms.[30] The reversibility of vestibular sensitivity and MRI enhancement is consistent with an inflammatory lesion of the VG.

The agents responsible for the ganglionitis are NT viruses, probably herpes simplex virus (HSV) or herpes zoster virus. Other NT viruses that may be included are cytomegalovirus, Epstein-Barr virus, and pseudorabies. The ubiquity of HSV is seen in the high exposure rates recorded in the world population.[31] Elevated serum antibody titers to HSV-1 have been recorded in 70% of 25-year-olds, and at the age of 60 years, the rate is more than 90%. After the NT virus enters a sensory nerve, it may reside in a latent state in its ganglion cells. Reactivation of the virus from latency, triggered by extreme stress, is reflected by clinical signs and symptoms.

Other investigators[11,13,32,33] have suggested primary vestibular nerve inflammation by viral agents in MD. However, the evidence presented was clinical association,[32,33] equivocal morphological description of viral inclusions in the VG,[11] and nonspecific degeneration with fibrosis in the vestibular nerve.[13] In addition, immunologic evidence supports the presence of NT virus in MD.[34-37] Despite an increasing body of evidence supporting viral vestibular ganglionitis in MD and VN, the concept of reactivation of latent virus in the VG causing asymmetry (vertigo) has not gained wide acceptance.

Support for viral vestibular ganglionitis in recurrent vestibulopathies such as MD, VN, and BPV has lacked 1) morphological evidence of VG damage indicative of viral ganglionitis and 2) identification of viral antigen or nucleic acid in the VG of patients with MD and VN. However, this support is now available. Pitovski et al[38] demonstrated HSV latency-associated transcripts and thymidine kinase in vestibular nerves excised surgically to relieve vertigo in MD patients. Arbusow et al[39] have amplified with polymerase chain reaction HSV-1 nucleic acid in 60% of VGs from 35 TBs derived at autopsy. A pattern of VG degeneration similar to the animal model of herpetic ganglionitis and human herpes zoster was observed in TBs from patients with VN, MD, and BPV.[40,41]

We believe that the presence of this form of VG degeneration in MD, VN, and BPV supports the concept of intermittent reactivation of latent NT virus in the VG as the pathogenesis for recurrent vertigo. Morphological and clinical evidence is presented to validate this hypothesis.

MATERIALS AND METHODS

The histopathologic findings in TBs from 3 patients with antemortem diagnoses of MD, VN, and BPV are presented to illustrate the pathological changes in the facial and vestibular nerves. The sensory ganglia of the facial nerve (geniculate and meatal) are important foci for NT virus invasion. These ganglionic masses were quantitatively described in 1998 in a series of 100 human TBs.[42] They are derived from different embryological anlagen: the geniculate ganglion (GG) from the epibranchial placode of the second branchial arch, and the meatal ganglion (MG) from the neural crest primordium. In most TBs (88%), the GG contains most of the sensory neurons in the facial nerve, and the MG is small. In approximately 12% of facial nerves, the MG may equal or exceed the number of ganglion cells in the GG. In the 1998 study,[42] the number of neurons in the GG ranged from 66 to 4,017 (mean, 1,713), and the MG contained 0 to 2,764 cells (mean, 448). Fourteen percent of the GGs contained fewer than 1,000 neurons, and 88% of the MGs contained fewer than 1,000 cells. Sixty-four percent of the MGs held fewer than 500 cells, and 34% had fewer than 200. The MGs represented the entire ganglion population of the facial nerve in 2% of the TBs.

Histopathologic changes in the facial and vestibular nerves of 75 human TBs were reviewed.[40] The TBs had been prepared in standard fashion after 10% formalin fixation, decalcification, and celloidin embedding. Horizontal sections cut at 20 μm were collected, and every 10th section was mounted, stained with hematoxylin and eosin, and cover slipped. Volume fractions of the degeneration in the MG and vestibular nerve were measured by a point-counting technique based on the Cavalieri method.[43] Fifty-one TBs were selected on the basis of degenerated neurons in the vestibular and facial nerves. In 20 of these TBs, a history of recurrent vertigo was present. A diagnosis of VN was made in 5 donors, of MD in 5 donors, and of BPV in 3 donors. A precise diagnosis of the vestibulopathy was not made in 7 TBs.

The remaining 24 TBs did not contain evidence of degeneration in the vestibular and facial nerves, but the patients were similar in age, sex, and other labyrinthine disorders. None of these 24 TBs were associated with a history of vertigo. These 24 repre-

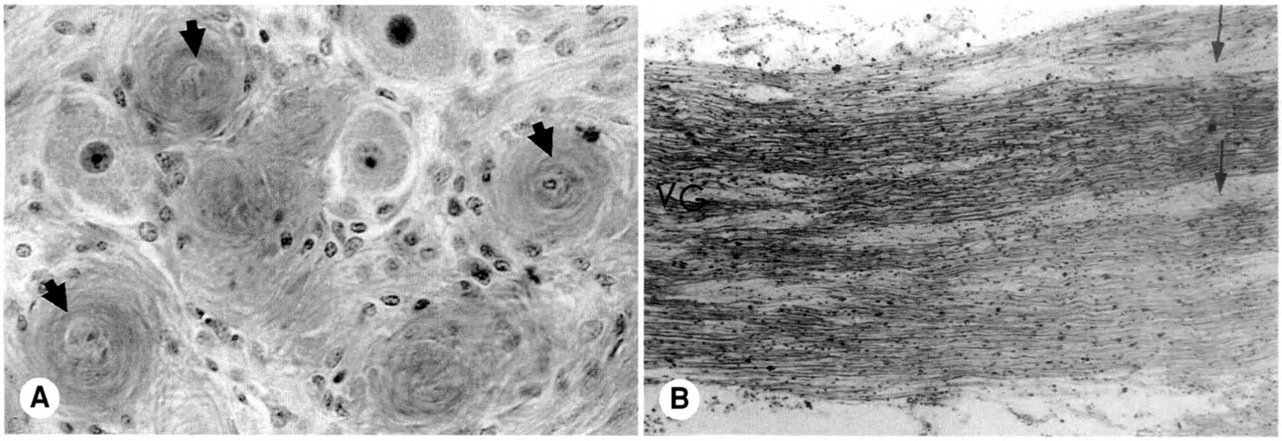

Fig 1. Histopathology of temporal bone from patient with vestibular neuronitis. **A**) Meatal ganglion shows several degenerated ganglion cells (arrowheads) and others surrounded by small, dark cells (original ×450). **B**) Vestibular nerve trunk holds numerous fascicles of degenerated axons (arrows; original ×64). VG — vestibular ganglion.

sent the control group.

A series of 45 consecutive patients were evaluated for a diagnosis of recurrent vertigo during a 15-week period (January 1, 1999, to April 15, 1999). They are included to demonstrate the co-occurrence of more than one vestibulopathy in the same ear and the co-occurrence of vestibulopathy with other cranial neuropathies.

RESULTS

A 71-year-old man with a 5-year history of prostate cancer with orbital and brain metastases was admitted to the hospital for severe vertigo and nausea. A hearing test showed a bilateral high-frequency sensorineural hearing loss with discrimination scores of 88% (right) and 68% (left). There was a spontaneous nystagmus to the right and a diminished caloric response on the left. He died 19 days later of brain stem and cerebellar infarction. On histopathologic examination of the left TB, the GG did not have degenerated neurons, but the ganglion cells were surrounded by an abundant number of satellite cells (SCs). In the MG, there were several scattered degenerated ganglion cells replaced by collagen-like material (Fig 1A). Intact ganglion cells were surrounded by many SCs. The cristae and maculae appeared to contain a normal number of hair cells and supporting cells. Several fascicles of axonal degeneration were observed in the superior vestibular nerve trunk (Fig 1B). Smaller fascicles of degenerated nerve fibers passed between neurons surrounded by many SCs and inflammatory cells (ICs) in the VG. Consistent with normal hearing thresholds and normal word discrimination for his age of 71 years, the organ of Corti was shrunken, but the pillar cells separated a normal number of internal and external hair cells. The organ of Corti was missing in the lower basal turn. The spiral ganglion cells appeared reduced in number, except for the extreme basal end of cochlea, where they were absent.

A 65-year-old man with alcoholism suffered a head injury that resulted in several hours of unconsciousness. Six months later, he was found to have post-traumatic normotensive hydrocephalus. During admission to the hospital, he complained of recurrent vertigo that had lasted for several months. The spells of vertigo lasted a few minutes and were associated with nausea and vomiting. There was a mild spontaneous nystagmus to the left and a tendency to fall to the left. Positional (Hallpike) tests revealed, in the left ear–down position, a rotatory clockwise nystagmus with a brief latency, a short duration, and fatigability on repeat testing. When the right ear was placed down, no nystagmus was observed. The findings on bithermal caloric stimulation were normal and symmetric. A hearing test revealed a high-frequency sensorineural hearing loss on the left. Speech discrimination was normal in both ears. Two months later, the patient died of pulmonary tuberculosis. On histopathologic examination of the left TB, the tympanic membrane, ossicles, and mastoid air cell system were normal. No evidence of TB fracture was present. There was a loss of hair cells in the basal turn of the cochlea (basal 6 mm) and atrophy of the stria vascularis in the upper turns of the cochlea. The spiral ganglion had a slight loss of neurons in the basal turn. The cupula of the posterior canal crista was reasonably well formed, but was shrunken and contained a few small basophilic deposits (Fig 2). The facial nerve MG contained a heavy infiltration of SCs that surrounded intact and degenerated ganglion cells (Fig 3). The ganglion of the superior vestibular division contained intact ganglion cells surrounded by a normal density of SCs (Fig 4A). No degenerated axons were detected in the superior vestibular nerve trunk. The inferior VG was composed of ganglion cells sur-

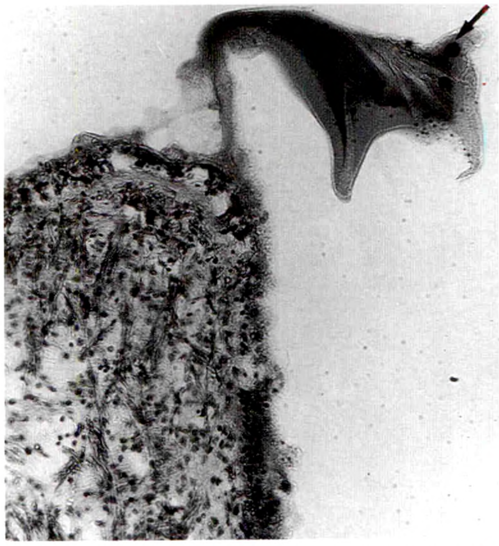

Fig 2. Cupula of posterior canal crista in left ear of patient with benign paroxysmal positional vertigo (BPV) contains round basophilic deposits (arrow) probably representing artifact (original ×100). (Reprinted with permission.[16])

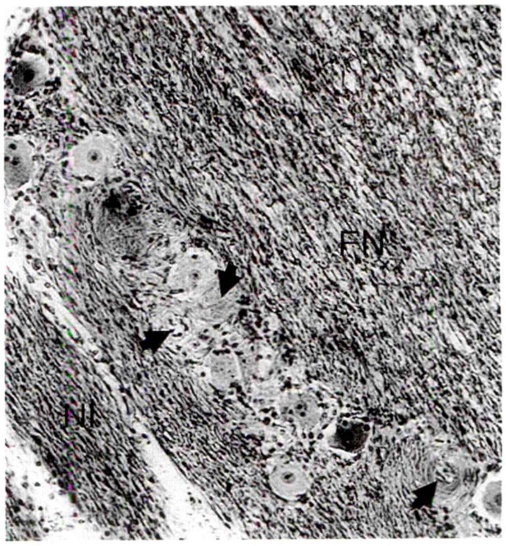

Fig 3. Patient with BPV (original ×280). Meatal ganglion of facial nerve (FN) contains degenerated (arrowheads) and intact ganglion cells surrounded by small dark cells. NI — nervus intermedius.

rounded by an increased number of SCs and ICs (Fig 4B). Several fascicles of degenerated axons in the inferior nerve trunk could be traced through the ganglion toward the singular nerve (Fig 5).

A 76-year-old woman had a slow progressive hearing loss for many years. An audiogram performed at 72 years of age revealed a bilateral severe sensorineural hearing loss with flat audiometric patterns at 70 dB on the right and 90 dB on the left. Her speech discrimination scores were 28% in the right ear and 0% on the left. At 73 years of age, she began to experience sudden episodes of vertigo with occasional falling. At 76 years of age, she died of cerebral hemorrhage and pontine infarction. On histopathologic examination of the right TB, there was severe EH of the labyrinth. Reissner's membrane was displaced to make contact with the walls of the scala vestibuli. There was atrophy of the stria vascularis in a patchy pattern throughout the middle and apical turns. There was a scattered loss of hair cells in the organ of Corti. The spiral ganglion was markedly reduced to 25% of normal. The saccule was distended, and the saccular wall was attached to the stapes footplate by a thick layer of fibrous tissue (Fig 6). There were outpouchings in the walls of the utricle and all 3 ampullae. The GG did not have any degenerated ganglion cells and had a normal density of SCs. The MG was filled with neurons surrounded by an abundance of SCs and several degenerated neurons (Fig 7A). The superior and inferior VG cells were surrounded by many SCs

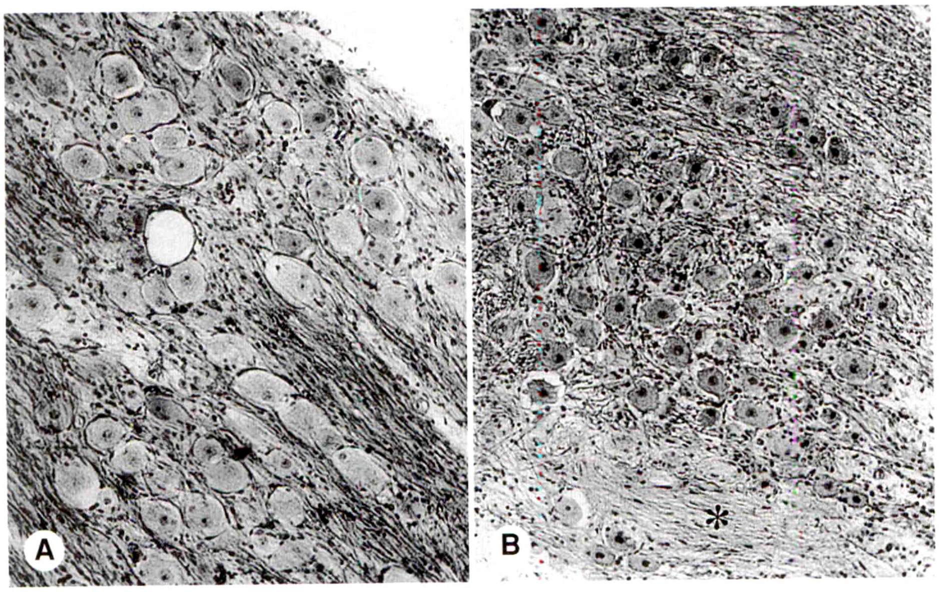

Fig 4. Patient with BPV (original ×280). **A)** Superior vestibular ganglion reveals normal density of satellite cells around ganglion cells. **B)** Inferior vestibular ganglion is densely populated with satellite and inflammatory cells. Bundle of degenerated axons traverses ganglion (asterisk). (Reprinted with permission.[16])

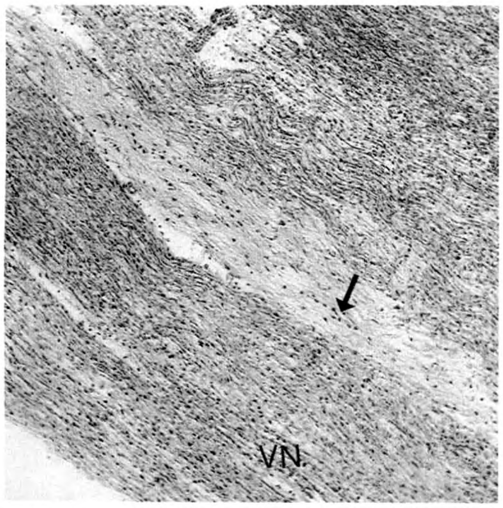

Fig 5. Patient with BPV (original ×80). Inferior vestibular nerve trunk (VN) contains fascicles of degenerated axons (arrow). (Reprinted with permission.[16])

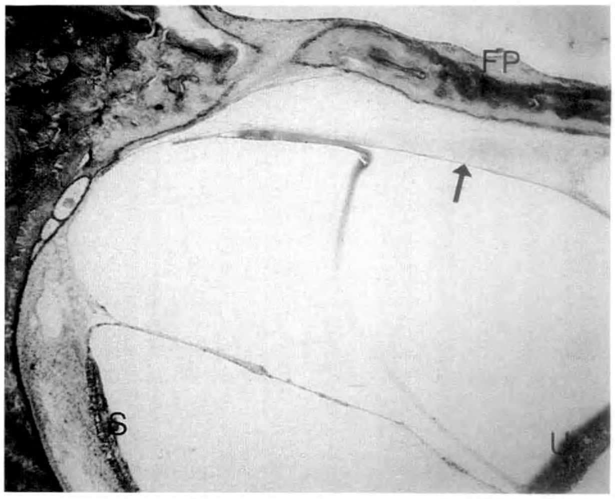

Fig 6. Patient with Meniere's disease (original ×80). Vestibule contains distended saccular wall attached to stapes footplate (FP) by dense layer of fibrous tissue (arrow). S — saccular macula, U — utricular macula.

and ICs. Small fascicles of degenerated axons were scattered throughout the vestibular nerve trunk (Fig 7B).

We studied 51 TBs that contained evidence of ganglion cell degeneration in the MG and VG. Although all of the VGs contained increased numbers of SCs and ICs around groups of ganglion cells, evidence of groups of degenerated neurons in the VG was found in only 5 TBs. However, 45 of the TBs contained focal axonal degeneration leading from the VG into the nerve trunk. Because the axonal projection of VG neurons is in a straightforward pattern in the vestibular nerve trunk, focal axonal degeneration represents degenerated fibers from groups of ganglion cells.[44]

In 25 TBs, focal vestibular axonal degeneration was associated with MG cell degeneration (Fig 8), whereas in 20 TBs with focal vestibular nerve degeneration, there was no MG degeneration (Fig 9). Increased SC and IC numbers surrounded MG neurons in the facial nerves of these TBs. Twenty of the 45 TBs were associated with a vertigo history: VN in 5, MD in 5, BPV in 3, and unknown vestibulopathy in 7. Although a history of vertigo was not recorded in more than half of these TBs, the occurrence of balance symptoms may be overlooked because of their solitary or episodic nature and a long interval before TB acquisition. In 6 TBs, there were degenerated neurons in the MG but none in the vestibular nerve; none of these patients had vertigo (Fig 10).

In a series of 24 TBs from individuals with similar age, sex, and otologic disorders and no history of

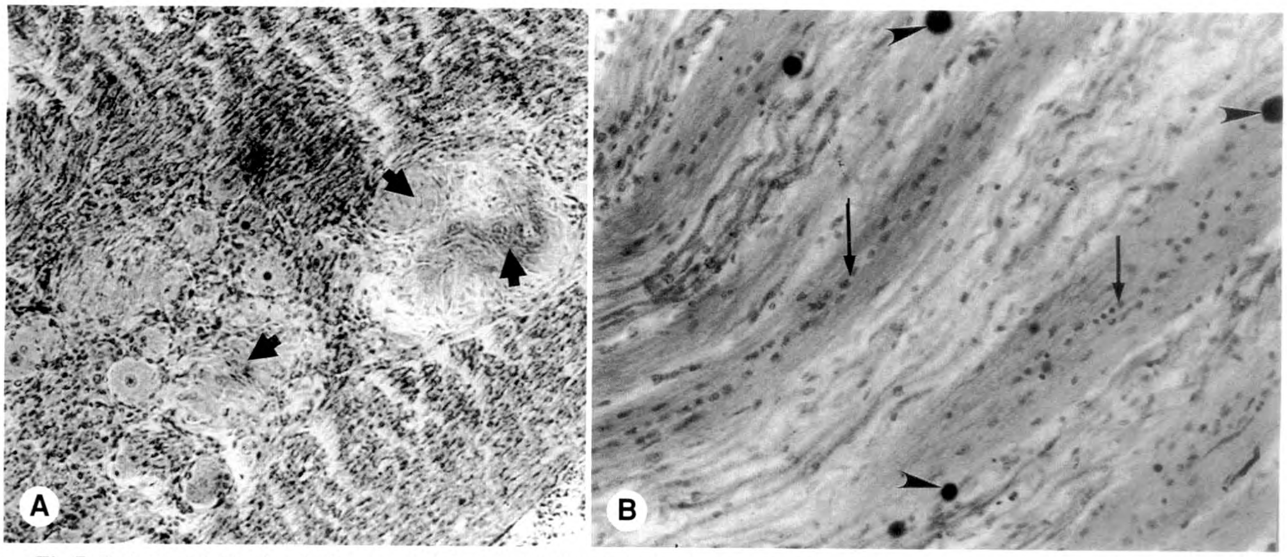

Fig 7. Patient with Meniere's disease. **A)** Meatal ganglion contains degenerated (arrows) and intact ganglion cells surrounded by small, dark cells (original ×280). **B)** Numerous fascicles of degenerated axons (arrows) can be seen in vestibular nerve trunk; arrowheads indicate deposits of lipid from degenerated myelin (original ×350).

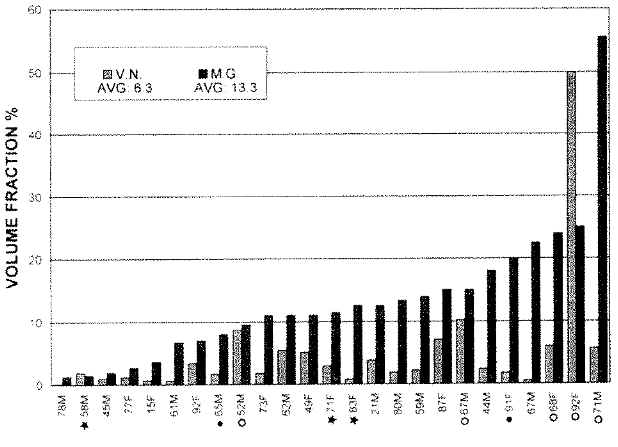

Fig 8. Graph shows volume fractions of degeneration in meatal ganglion (M.G.) and vestibular nerve (V.N.) of 25 temporal bones. Diagnosis of vestibular neuronitis (circles), Meniere's disease (stars), or benign paroxysmal positional vertigo (asterisks) was made in 10 temporal bones.

vertigo, there was no evidence of degeneration in the MG or vestibular nerve.[40]

In our clinical series, 45 consecutive patients were evaluated for recurrent vertigo during a 15-week period (January 1, 1999, to April 15, 1999; Table 1). There were 29 female and 16 male patients ranging in age from 31 to 93 years (mean, 62 years). Thirteen patients had MD and BPV in the same ear; 4 of these patients also gave a history of HSV labialis or herpes zoster on the side of the affected ear. Fourteen patients manifested VN and BPV in the same ear; 3 gave a history of HSV labialis on the side of the affected ear. All 9 patients with BPV alone gave a history of HSV labialis or HSV facialis on the side provoking vertigo. Five patients with a history of idiopathic facial paralysis developed VN (2 patients), BPV (2 patients), or MD (1 patient) in the same ear. One of these experienced sequential idiopathic facial paralysis and VN bilaterally. The 4 remaining patients in the series presented a single vestibulopathy (MD in 2 patients and BPV in 2 patients) without an associated cranial neuropathy. In 1 patient, the onset of vestibular symptoms followed surgery on the eye, and in another patient the onset followed a sinus infection.

In 2 patients, MRI of the TB had been performed by referring physicians. One, a 52-year-old woman, had a 2-year history of right aural fullness, fluctuating hearing loss, and recurrent vertigo (15 minutes to 1 hour in duration). The right ear had a low-frequency sensorineural hearing loss (40 dB) and 88% word discrimination, and the left ear had normal hearing. Electronystagmography demonstrated a 70% right canal paresis. An MRI scan with contrast revealed focal enhancement in the distal internal auditory canal (Fig 11A). A middle cranial fossa approach was used to excise the vestibular nerve and ganglion. The pathology report described neural tissue with ganglion cells surrounded by many small, dark cells and focal axonal fibrosis (Fig 11B). Immunoperoxidase staining with antibodies to HSV was positive in ganglion cells.

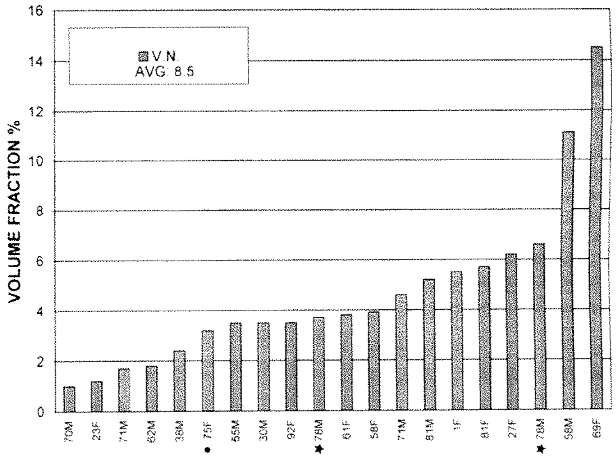

Fig 9. Twenty temporal bones contained measurable degeneration in vestibular nerve (V.N.) only. Diagnosis of Meniere's disease (stars) or benign paroxysmal positional vertigo (asterisk) was made in 3 temporal bones.

The other patient referred with MRI findings was a 45-year-old man with an 8-month history of recurrent vertigo (several hours' duration) without hearing loss who had positional vertigo (20 to 25 seconds) with the right ear down. Electronystagmography demonstrated a 32% right canal paresis and a positive right Hallpike positional test. The hearing was normal. An enhanced MRI scan revealed focal enhancement in the right internal auditory canal (Fig 12A). A repeat MRI scan performed 2 months later showed decreased enhancement in the right internal auditory canal (Fig 12B).

DISCUSSION

Neurotropic viruses are characterized by their ability to enter the terminus of a neuron and travel centripetally by axoplasmic transport to the cell body, in which they localize in the cytoplasm and nucleus.[45,46] After causing an active productive infection, the virion may leave the cell body or assume a latent state within the nucleus, in which it is isolated from circulating antibodies. When virus is released from the cell during active infection, it enters the extracellular space between neurons and SCs. The virus is internalized by an increased number of SCs, and its spread to adjacent neurons over tight junctions in the cell membrane or by SCs results in a cluster-like arrangement of infected neurons.[45]

The ganglion cell degeneration in the MG and the

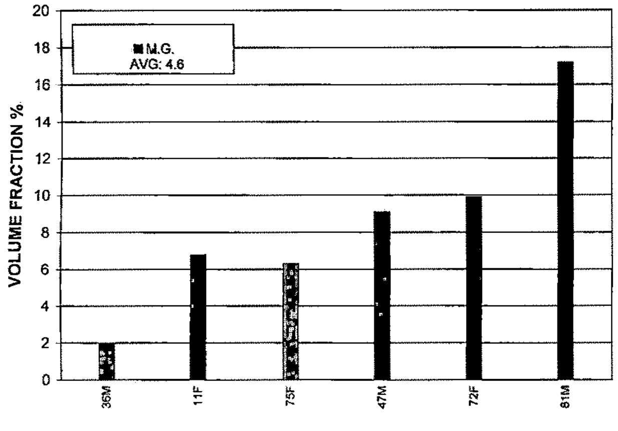

Fig 10. Degeneration in meatal ganglion (M.G.) only was measured in 6 temporal bones, none of which carried vestibular diagnosis.

VG can be divided into 3 phases consistent with herpes virus infection. The first of these stages represents the active productive phase of herpetic ganglionitis, which has been described in mice.[46] After exposing the abraded hind footpads of the mouse to HSV, Cook and Stevens[46] found mature virus particles in and around sacral spiral ganglion cells after 20 to 24 hours. The histologic picture in the ganglion during the 5 to 6 days in which virus could be detected was characterized by a generous surround of SCs, macrophages, and lymphocytes (Fig 13[46]). Virus-specific immunofluorescence was found in both neurons and SCs. At day 6, ganglion cells were vacuolated and surrounded by nests of round cells. The inflammatory response gradually diminished over 4 weeks. Animal models of the degenerative stages of herpetic ganglionitis are not available because of the long-term nature of such studies. On the basis of examples of degenerating vestibular and meatal ganglion cells in these TBs, we suggest the illustrated degenerative stages of herpetic ganglionitis (Fig 13). The inferior VG in the TB of a 75-year-old woman with BPV (Fig 14) demonstrates the transition from ganglion cells surrounded and replaced by SCs to those being replaced by collagen-like material. Concentric replacement of ganglion cells with collagen-like substance is also found in the MG (Fig 1A). Because this proliferation of SCs results in increased membrane apposition, the deposition of laminated collagen-like substance is a likely result.

TABLE 1. POLYNEUROPATHY IN 45 PATIENTS WITH RECURRENT VERTIGO

Vestibular Nerve (n)	*Facial Nerve*	*Trigeminal Nerve*
MD + BPV (13)	0	4
VN + BPV (14)	0	3
BPV (9)	0	9
IFP + VN (2)	2	0
IFP + BPV (2)	2	0
IFP + MD (1)	1	0
MD (2)	0	0
BPV (2)	0	0

MD — Meniere's disease, BPV — benign paroxysmal positional vertigo, VN — vestibular neuronitis, IFP — idiopathic facial paralysis.

The SC has long been thought to be intimately related to its ganglion cell. Satellite cells may support the neuron metabolically during prolonged activity. This suggestion is supported by decreased nucleic acid content in SCs, whereas neuronal nucleic acid is increased in the superior cervical ganglion after prolonged (3-hour) stimulation.[47] Furthermore, SCs proliferate in response to increased metabolic or synaptic activity.[48] The role of SCs in the NT viral infection of a ganglion may represent a response to increased neural activity, as well as the need to limit the spread of virion release from ganglion cells.

The host neuron also affects the mode of NT virus presence in the cell. Margolis et al[49] presented evidence that 2 types of neurons in the mouse dorsal root ganglion allow different forms of virus infection after a single inoculation of virus. In one population of ganglion cells, viral protein synthesis was high. But synthesis of latency-associated transcripts was minimal, whereas in a second neuronal type, viral gene expression was restricted, but synthesis of latency-associated transcripts was abundant. This finding suggests that after uptake of NT virus, latent infection will occur in one type of ganglion cell and active productive infection will occur in another type of ganglion cell within the same ganglionic mass.

The observation of degenerated ganglion cells in the MG component of the facial nerve in many of the vestibulopathic TBs, while degenerating ganglion cells were found in the VG of only 5 TBs, may be a reflection of the pathological response to virus in ganglion cells of different embryological derivations. The MG develops from a neural crest origin, whereas the VG is derived from the auditory placode.[42] However, focal axonal vestibular nerve degeneration represents indirect evidence of ganglion cell loss in clusters. The frequent association of degeneration in these two ganglionic masses in the internal auditory canal suggests a causal relationship.

If the virus assumes a latent state after initial infection of the neuron, it may be reactivated and replicated at some later time by a stressful event. Epinephrine has been shown to cause reactivation of latent HSV in mouse sensory ganglion.[50] Latency may mean that the viral genome is maintained in a subviral state in both ganglion cells and SCs. Activation from latency means replication of the virus and its nucleic acids. When activated, the incomplete virus

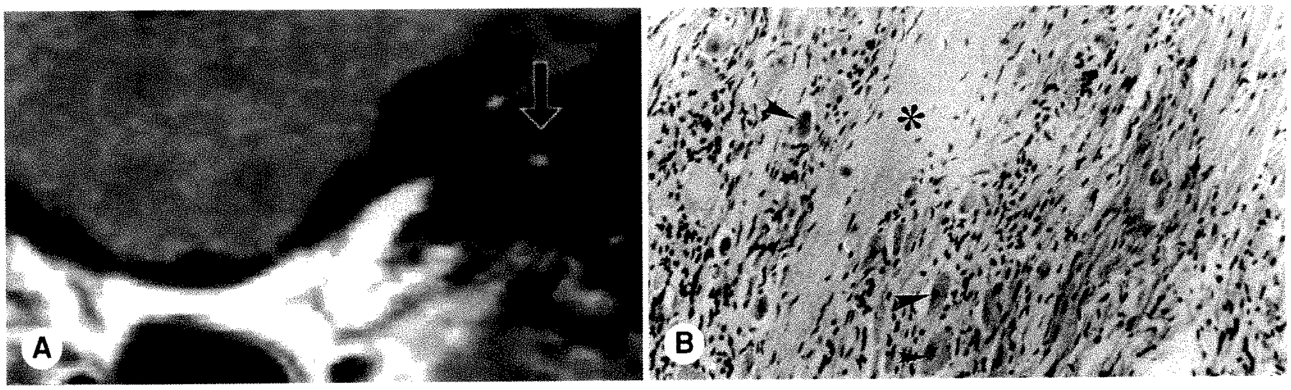

Fig 11. Patient from clinical series (52-year-old woman). **A)** Magnetic resonance image shows focal enhancement (arrow) within right internal auditory canal. **B)** Excised vestibular ganglion contains ganglion cells surrounded by many satellite and inflammatory cells (original ×200). Focal axonal degeneration (asterisk) is seen between ganglion cells (arrowheads).

core acquires a double envelope of lipoprotein from the nuclear and cell membranes before its release through breaks in the cell membrane.[51] Virus in the extracellular space may be neutralized by circulating antibodies. Infectious nucleic acids, on the other hand, are neutralized by nuclease enzymes released by blood components.[52] When the NT virus is reactivated, it travels by axoplasmic transport along the cell appendages. If the transport is anterograde, the virus is carried toward the central nervous system; retrograde transport will carry the virus in the peripheral nerve branches to the sense organ. The direction of intra-axonal flow is dependent on the virus strain, especially with HSV.[53-55] The H129 strain of HSV-1 is carried preferentially in an anterograde direction, whereas the McIntyre B strain follows a retrograde direction of flow to the periphery.[53,55,56] This directionality of flow determines to some degree the clinical expression of NT viral ganglionitis.

The mechanism by which vestibular neuronal activity is altered to produce vertigo is not known. However, virus activation and release into the extracellular space disrupts the ganglion cell membrane, with leakage of ionic constituents across the cell wall. Because neuronal excitability is dependent on the ionic gradient across the cell membrane, loss of this gradient by flow of potassium ion to the outer coat of the membrane, in which they displace bound calcium ion, is a possible explanation.[57] Recurrent disruption of the ganglion cell membrane may eventually cause neuronal death.

A viral vestibular ganglionitis has long been considered the probable cause of VN. Epidemiological risk factors and elevated viral (HSV) serum antibodies have been described in clinical series of VN.[19,20,22] Reported TB findings[25,26] from patients who fit the accepted criteria for VN include advanced degeneration of the VG and peripheral dendrites with sparing of the auditory nerve and sense organ. The current requirement of total or near-total loss of vestibular response in one or both ears for a diagnosis of VN[22] is probably too restrictive. Vestibular hypofunction greater than 20% supports a diagnosis of VN. Approximately 50% of patients with acute VN show reversibility of decreased vestibular function with recovery.[26,28] Such recovery of a decreased vestibulo-ocular reflex is consistent with an inflammatory process in the VG. Enhancement of the VG on MRI has been reported in VN[30] and MD (unpublished observations). In the early phases of VN, minimal neural

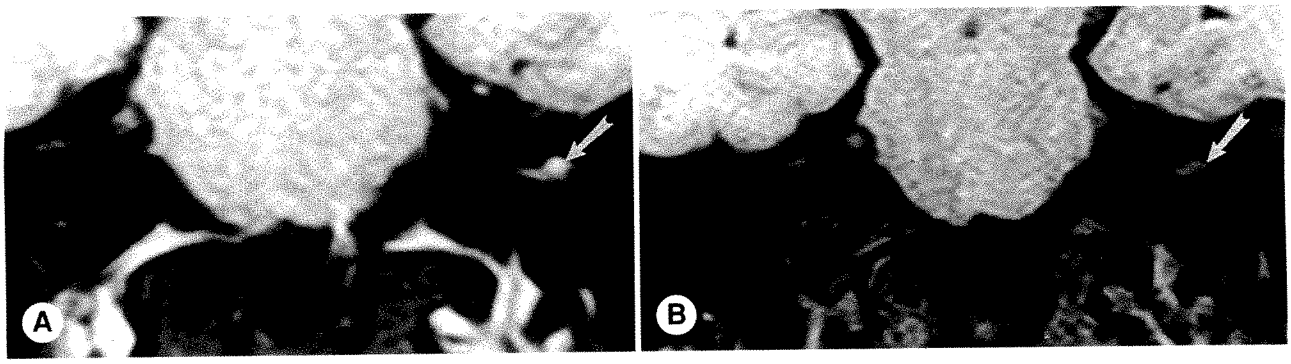

Fig 12. Patient from clinical series (45-year-old man) who had vestibular neuronitis. **A)** Magnetic resonance image shows localized enhancement in internal auditory canal (arrow). Note tail on enhanced area. **B)** Two months later, enhancement has decreased (arrow).

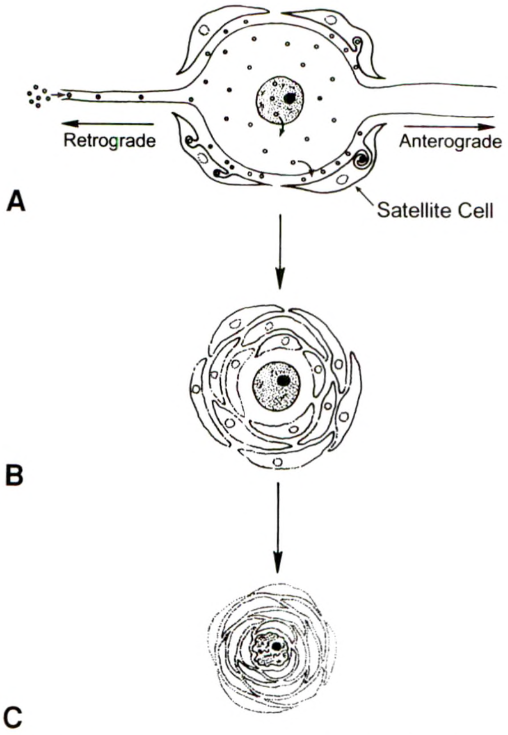

Fig 13. Schematic shows morphological changes in sensory ganglion cell with herpetic infection. **A)** Active productive infection according to Cook and Stevens.[46] **B,C)** Suggested changes that lead to degeneration.

degeneration may not be sufficient to produce a decrease in the response to caloric stimulation. Failure to demonstrate viral antigen in the VG does not argue against a viral ganglionitis, as viral nucleic acids may be responsible for the infectious process.[52] Morphological changes in TBs from such patients indicate a neuronal degenerative lesion.[40] The same-sided association of MD and BPV with other viral cranial neuropathies (idiopathic facial paralysis, trigeminal HSV, VN) supports the concept of a viral (HSV) polyganglionitis as proposed by Adour et al.[32]

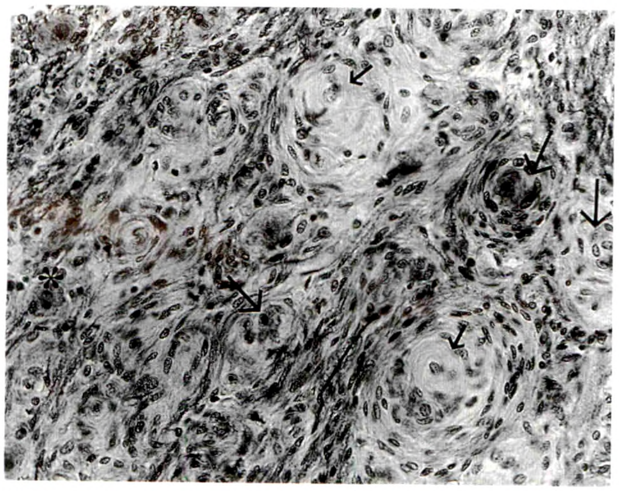

Fig 14. Inferior vestibular ganglion of 75-year-old woman with benign paroxysmal positional vertigo (sixth patient on Fig 9) demonstrates transition from ganglion cells surrounded by satellite cells (large arrows) to ganglion cells replaced by satellite cells (small arrows; original ×450).

Although head trauma is frequently associated causally with the onset of BPV, a common preceding event to BPV is an acute viral vestibular syndrome (VN or neurolabyrinthitis) associated with an upper respiratory tract infection. The BPV becomes evident when the traumatic event or vestibular syndrome has subsided.[15] The onset of BPV may also follow surgery under general or spinal anesthesia (heart surgery, abdominal, gynecologic, dental, lumbar).[58] Early TB reports of BPV described extensive degeneration of the superior VG with intact innervation to the saccule and the posterior canal crista. The combination of a degenerated superior VG and an intact inferior VG in the same ear constitutes the pathological correlate for coexistent VN and BPV.[59] This correlate was used to support the contention that the posterior canal crista is responsible for the rotatory nystagmus and vertigo that may be provoked by the ear-down or head-back position. The search for the mechanism of posterior canal crista activation in BPV culminated in the cupulolithiasis concept.[14]

Parnes and McClure[60] introduced a modification of this concept. On the basis of intraoperative observations that particles resembling otoliths float freely or in clumps in the endolymph of the posterior canal crista, they suggested that these gravity-sensitive otoliths create a drag on endolymph flow, leading to cupular deflection during head movement. This idea has been organized into the concept of canalolithiasis, which has been used to explain the spontaneous remissions and exacerbations of BPV in many patients. However, this mechanical concept does not account for the short duration (15 to 25 seconds) of nystagmus in the presence of sustained provocation, the fatigability of nystagmus on repeat provocation, and the prolonged (months to years) spontaneous remissions between periods of BPV. Morphological changes indicating acute (SC increase) and chronic (axonal degeneration) viral ganglionitis in the inferior VG point to a neural component in BPV.[16] Reactivated virus may change the electrical and ionic balance gradient across the membrane of posterior canal crista ganglion cells, especially that of calcium ion, rendering the cells hyperexcitable and fatigable when provoked.[57] Relief of BPV follows ablation of these sensitized neural units (singular neurectomy) or hair cells that initiate the neural spike (posterior crista canal occlusion).

Meniere's disease had long been considered to be a result of decreased endolymph resorption leading to EH. This concept is based on the repeated descriptions of EH in TBs from patients with MD and the

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creation of EH following endolymphatic duct obstruction in some laboratory animals. Behavioral and histopathologic differences between the animal models of EH and MD patients have been pointed out earlier. Attempts to relieve EH by enlarging the endolymphatic sac or by reducing body fluid have had equivocal success in controlling vertigo and hearing loss in MD.

Recently reported TB evidence supports a concept of MD as a manifestation of vestibular ganglionitis.[61] Focal axonal vestibular nerve degeneration with SC and IC increase in the VG was found in TBs of MD patients. The concept of VG viral neuropathy is reinforced by the report of Pitovski et al[38] that described latency-associated transcript and thymidine kinase genes in VGs excised from MD patients. These findings are strong support that MD is an expression of vestibular ganglionitis caused by an NT virus, likely of the Herpes virinae family.

Viral vestibular ganglionitis is an appropriate basis for the signs and symptoms of MD. The onset of MD in adulthood or middle age is similar to that of other viral neuropathies (idiopathic facial paralysis, VN, BPV) and is often triggered by a stressful event (head trauma, viral labyrinthitis in childhood, or migraine headache). Meniere's disease is usually unilateral, with an incidence of bilaterality ranging from 15% to 50%. Reactivation of latent virus in the VG can account for episodes of vertigo of various durations and intervals. Reactivated HSV from the VG is carried by retrograde flow in vestibular nerve branches to the sense organs. The symptoms and pathological features of MD result from a recurrent labyrinthitis caused by the release of viral protein or nucleic acid into the perilymphatic compartment. Fibrosis in the vestibular cistern,[62] the scala vestibuli, and the posterior canal crista ampullary recesses in TBs from MD patients represents a response to the infectious virus or nucleic acid. Retraction of this fibrous tissue response may displace the saccular wall and Reissner's membrane and cause outpouchings of the vestibular labyrinth, giving the appearance of EH. Such displacement of yielding membranes in the labyrinth was observed by Wittmaack[63] after the induction of serous labyrinthitis in laboratory animals.

The fluctuating sensorineural hearing loss that occurs in MD probably represents neurotoxic effects on the SG rather than mechanical alteration of the motion mechanics of the basilar membrane. Characteristic of the low-frequency sensorineural hearing loss found in early MD is the upward slope of elevated thresholds for low frequencies to a normal response at 1 kHz and above. Elevation of thresholds caused by mechanical damping of the basilar membrane with increased endolymph should follow an even gradient of threshold elevation from the lowest to the highest frequency, because of a gradient of the physical characteristics of the basilar membrane and associated structures. In MD, the envelope of basilar membrane excitation initiated by a low-frequency stimulus travels toward the base and is recorded by intact basal ganglion cells at 1 kHz. The early involvement of low frequencies and later involvement of all frequencies in the clinical course of MD is explained by a neurotoxic effect on SG cells by toxic proteins or nucleic acids that reach the scala tympani. This effect would be first seen in apical ganglion cells, as contaminated perilymph travels from the scala vestibuli through the helicotrema and into the scala tympani, from which it can flow through perforating canaliculi in the osseous spiral lamina to reach dendrites of the apical SG and result in a low-frequency auditory threshold elevation. Repeated toxic insults could account for fluctuations in threshold and word discrimination, eventually producing ganglion cell loss in the apical turn.[64] Continued viral release may eventually extend down the scala tympani to affect more basal segments of the SG, producing an even threshold elevation for all frequencies in advanced MD.

It is difficult to explain the delayed forms of EH (ipsilateral and contralateral) on the basis of reduced endolymph absorption. However, reactivation of a latent vestibular ganglionitis may account for the clinical presentation of vertigo and/or hearing loss ipsilateral or contralateral to an inner ear damaged by viral labyrinthitis more than 20 years earlier. Vestibular sensitivity has been noted to be profoundly reduced in the deafened ear with ipsilateral delayed EH and in both ears with contralateral delayed EH.[65,66] This finding suggests that contralateral delayed EH represents bilateral NT vestibular ganglionitis, whereas ipsilateral delayed EH is produced by a unilateral ganglion infection.

The co-occurrence of more than 1 vestibulopathy in the same ear has been noted by several investigators. The finding of BPV in the affected ear of 14 patients with VN and in the affected ear of 13 patients with MD in our series of 45 patients is best explained by a similar disorder located in the VG. Furthermore, the appearance of VN, MD, or BPV after idiopathic facial paralysis in the same ear is suggestive of a similar disorder. These clinical associations are most logically explained as an inflammatory process involving adjacent anatomic structures.

CONCLUSION

These observations are presented to support the view that the syndromes of VN, BPV, and MD are

TABLE 2. CLASSIFICATION OF RECURRENT (VIRAL) VESTIBULOPATHIES

- Anterograde virus strain (hearing preserved)
  - Superior vestibular ganglionitis (VN, vestibular MD)
  - Inferior vestibular ganglionitis (BPV)
  - Superior and inferior vestibular ganglionitis (VN and BPV)
- Retrograde virus strain (hearing affected)
  - Superior vestibular ganglionitis (MD, neurolabyrinthitis)
  - Subtype, utricular ganglionitis (Tumarkin's otolithic crisis)
  - Superior and inferior vestibular ganglionitis (MD and BPV)

clinical expressions of vestibular ganglionitis probably caused by the alpha Herpes virinae family. Several factors may determine the "face" presented in individual patients. These are 1) the amount of virus present (viral load), 2) the virus type and strain, 3) the location and number of affected VG cells, and 4) host resistance. It is possible that other forms of recurrent vertigo are expressions of vestibular ganglionitis. Innovative clinical terms have been used to describe these unusual presentations (ie, atypical VN, recurrent vestibulopathy, idiopathic vestibulopathy, and psychogenic vestibulopathy). The correlation of clinical and functional deficits with histopathologic changes may be used to classify vestibular disorders caused by vestibular ganglionitis. The portion of the VG affected and the type of NT virus responsible are 2 major descriptors in such a classification (Table 2).

According to this system, the vestibular syndromes are divided according to the presence of hearing loss, which is dependent on the strain of virus. The subtypes within the major classes are dependent on the location and number of affected ganglion cells in the VG. In parentheses are the currently recognized syndromes of vestibulopathy that result from the various locations of vestibular ganglionitis.

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SUDDEN DEATH AFTER NECK DISSECTION FOR CANCER

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The goal of this study was to analyze the mortality data following neck dissection and determine the risk factors of early death. The hospital mortality records were analyzed from 3,015 consecutive patients who underwent neck dissection. A case control study analyzed risk factors of death during the first 3 postoperative days. The mortality incidences were 0.50% and 1.33%, respectively, during the first 3 and the first 30 postoperative days. Eleven of the 12 unexplained deaths occurred during the first 3 postoperative days, and most of these patients died suddenly. They were more likely to be alcoholic and to have undergone nerve section. In most of the patients who died after the third postoperative day, death was related to a postoperative complication. Although the mechanisms of sudden death remain unclear, careful follow-up of these patients during the early postoperative days should be performed to reduce the mortality risk by shortening the delay of care.

KEY WORDS — mortality, neck dissection.

INTRODUCTION

Neck dissection is a common procedure performed in the treatment of head and neck cancers, because recurrence is frequent in the lymph nodes.[1]

Specific postoperative complications (mainly cardiovascular and respiratory) have been reported. The postoperative mortality incidence differs among studies (1.2% to 12% at 30 days),[2-6] and the exact causes of death are not always clearly identified. The aim of this study was to analyze causes and risk factors of death after neck dissection.

The postoperative mortality data were analyzed retrospectively for the first 30 postoperative days in 3,015 patients treated between 1980 and 1993. Then, risk factors of early postoperative death (from day 0 to day 3) were analyzed by a case control study.

PATIENTS AND METHODS

Patients who underwent neck dissection for cervicofacial cancer from 1980 to 1993 were included. Procedures for head and neck cancer were performed by the surgeons of the otolaryngology department. Procedures for melanoma and thyroid cancer were performed by the surgeons of the general surgery department. The period of the study extended up to the first 30 days after surgery. Most of the patients were hospitalized in the intensive care unit during the first night after surgery. The circumstances and cause of death were identified from medical and nursing records and postmortem examination.

To determine the risk factors of early death, a case control study was undertaken on patients who died during the first 3 postoperative days. Five patients had a postmortem examination. For each deceased patient, 3 control subjects were matched on 3 criteria: sex, age, and date of surgery. (The case control patient underwent neck dissection before the date of surgery of the deceased patient.)

Preoperative conditions were determined mostly from clinical records: patient height and weight, hypertension, myocardial ischemia, left ventricular failure, thromboembolic disease, asthma, chronic obstructive pulmonary disease (COPD), allergy, alcohol intake, smoking habits shown by number of packs per year, preoperative drug treatment, preoperative radiotherapy or chemotherapy, renal or hepatic disease detected from blood tests, preoperative serum potassium, and electrocardiography (EKG; P-R and Q-Tc intervals expressed in seconds; whether the EKG results were normal or abnormal; presence of ischemia or rhythm or conduction abnormalities).

The tumors were staged by the International Union Against Cancer TNM classification (tumor size divided into 3 classes: T0, T1 + T2, T3 + T4; lymph node involvement divided into 3 classes: N0, N1 + N2, N3 + N4; and presence of metastases), with the

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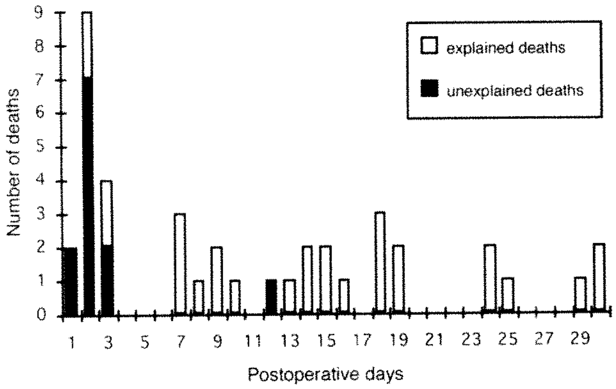

Postoperative mortality data after neck dissection (3,015 patients operated on between 1980 and 1993). Most unexplained deaths occurred before fourth postoperative day.

localization divided into 3 classes (hypopharynx or larynx; oropharynx or nasopharynx; no cancer, primitive, or other localization).

From the surgical and pathology reports, we collected data on dissection side (right, left, or bilateral), surgical procedure divided into 3 classes (hypopharynx or larynx, oropharynx or nasopharynx, surgery without mucosal opening), internal jugular or external carotid ligature, nerve X, XI, or XII section, duration of operation, perioperative blood transfusion, tracheotomy, and existence of lymph node capsule rupture.

For the postoperative period, reoperation, anticoagulant administration or opiate analgesic regimen, serum potassium, urea and creatinine levels, and EKG (normal or abnormal, P-R and Q-Tc intervals expressed in seconds) were considered.

Student's *t*-test was used for continuous variables with a normal distribution, and a χ^2 test and Fisher's exact test were used for discrete variables. When the distribution was not normal, nonparametric tests were chosen. A value of $p < .05$ was considered as the threshold of significance.

RESULTS

In the interval between 1980 and 1993, 3,015 patients underwent neck dissection. The number of deaths after neck dissection according to the postoperative interval is shown in the Figure. The overall 30-day postoperative mortality rate was 1.33% (40 deaths). The mortality rate peaked during the first 3 postoperative days (15 patients out of 40; see Figure). The causes of death are listed in Table 1. Airway obstruction occurred in 3 patients without tracheotomy, and intracranial hypertension after bilateral neck dissection was confirmed by computed tomography. Eleven of the 12 unexplained deaths (including 4 with postmortem examination) occurred during the first 3 postoperative days.

TABLE 1. CAUSES OF DEATH ACCORDING TO TIME PERIOD

Cause	*Days 0 to 3*	*Days 4 to 30*
Myocardial infarction	0	2
Pulmonary embolism	0	2
Pulmonary infection	0	5
Asthma attack	1	0
Tracheal bleeding	1	0
Airway obstruction	2	1
Stroke	0	2
Intracranial hypertension	0	1
Cervical bleeding	0	7
Metastasis	0	4
Unexplained	11	1

The overall 3-day postoperative mortality rate was 0.50%, with no operative deaths. The cause of death was determined for 4 patients (clinically for 3 and by postmortem examination for 1; Table 1). For 11 of the 15 patients (73%), neither the clinical history nor postmortem examination (5 patients) allowed determination of the cause of death.

There was no statistical difference for studied factors between the group with unexplained deaths and the group with explained deaths. Nevertheless, the 3 patients with vagus nerve section were in the group of unexplained deaths; they were not tracheotomized, and 2 of them had postmortem examinations, which did not find any explanation for the deaths.

In the case control study, no significant difference was found between the group of deceased patients and the control group with regard to preoperative blood sample, EKG findings, or preoperative risk factors, except for alcohol intake (Table 2). The other risk factor for early postoperative death was nerve section (Table 3; X: 3/12 versus 0/44, $p = .014$; XI: 9/6 versus 11/33, $p = .025$; XII: 2/13 versus 3/44, not significant). There was no significant prolongation of the Q-Tc interval in the group of deceased patients.

DISCUSSION

Although intraoperative and postoperative care changed over the 14 years encompassed in the study, the incidence of mortality did not change during this period. Lack of a precise severity score including both cancer stage and medical history impedes any conclusion.

The main results of these studies were that 1) there was a peak in mortality rate during the first 3 postoperative days, and 2) this peak was in relation to a high incidence of sudden death that was unexplained either by clinical examination or by postmortem examination. This peak in mortality during the first 3

TABLE 2. PREOPERATIVE DATA FOR DECEASED VERSUS CASE CONTROL PATIENTS

	Deceased	*Control*
Age (y)	64 ± 8 (15)	61 ± 8 (45)
Sex (M/F)	12/3	33/12
Height (cm)	168 ± 7 (12)	170 ± 9 (38)
Weight (kg)	68 ± 13 (14)	69 ± 14 (44)
Tobacco (pack-years)	42 ± 31 (9)	41 ± 27 (33)
Alcoholism (Y/N)*	12/0	27/13
Hypertension (Y/N)	4/11	11/33
Myocardial ischemia (Y/N)	3/12	5/40
Cardiac insufficiency (Y/N)	1/14	0/45
Chronic obstructive pulmonary disease (Y/N)	5/10	8/37
Asthma (Y/N)	1/14	1/44
Allergy (Y/N)	2/13	5/40
Cardiac or respiratory treatment (Y/N)	11/5	31/14
Preoperative radiotherapy (Y/N)	5/9	7/38
Preoperative chemotherapy (Y/N)	4/11	12/33
Preoperative potassium level (mmol/L)	4.1 ± 0.4 (15)	4.2 ± 0.5 (44)
Preoperative gamma glutamyl transferase (U/mL)	102 ± 152 (13)	45 ± 37 (40)
Preoperative aspartate aminotransferase (U/mL)	33 ± 25 (13)	24 ± 19 (39)
Preoperative alanine aminotransferase (U/mL)	31 ± 29 (13)	23 ± 24 (39)
Preoperative urea (mmol/L)	3.9 ± 1.7 (15)	4.6 ± 2.5 (45)
Preoperative creatinine (μmol/L)	76 ± 14 (15)	81 ± 22 (44)
Normal preoperative electrocardiogram (Y/N)	8/7	19/25
Preoperative P-R interval (s)	0.16 ± 0.03 (15)	0.16 ± 0.03 (41)
Preoperative Q-Tc interval (s)	0.43 ± 0.03 (15)	0.42 ± 0.04 (40)

Data are mean ± SD, or numbers of patients. Numbers in parentheses are numbers of patients contributing to mean.

*p = .02. No other differences were significant.

TABLE 3. TUMOR CHARACTERISTICS AND SURGICAL PROCEDURES IN DECEASED VERSUS CASE CONTROL PATIENTS

	Deceased	*Control*
Epidermoid cancer (Y/N)	15/0	40/5
Tumor size (T0/T1-2/T3-4)	2/8/6	0/22/21
Node size (N0/N1-2/N3-4)	5/7/3	20/15/8
Metastasis (Y/N)	0/15	0/43
Capsule rupture (Y/N)	11/4	22/21
Localization (A/B/C)*	3/5/7	7/13/25
Nasogastric tube (Y/N)	6/8	28/14
Tracheotomy (Y/N)	10/5	34/11
Neck dissection side (L/R/bilateral)	7/5/3	13/13/19
Surgical procedure (A/B/C)†	6/4/5	12/12/21
External carotid ligature (Y/N)	3/12	5/39
Carotid adhesion (Y/N)	1/14	1/42
Internal jugular ligature (Y/N)	8/7	16/28
Nerve section (Y/N)§	9/6	12/32
Surgery time (h)	5.8 ± 2.3 (14)	5.6 ± 1.9 (45)
Reoperation (Y/N)	1/14	1/43
Postoperative infection (Y/N)	0/15	4/40
Blood transfusion (L)	0.6 ± 1.0 (14)	0.4 ± 0.8 (45)

Data are mean ± SD, or numbers of patients. Numbers in parentheses are numbers of patients contributing to mean.

*A — T0 or not epidermoid cancer; B — nasopharynx or oropharynx; C — hypopharynx or larynx.

†A — no surgery on upper airway; B — nasopharynx or oropharynx; C — hypopharynx or larynx.

§p = .03. No other differences were significant.

days after neck dissection has not yet been reported. The 30-day mortality rate in our study was similar to that of a recent large study of laryngectomized patients.[3] Most of the unexplained deaths occurred during this period, but the low number of postmortem examinations may partly explain the high incidence of unexplained death. Nevertheless, postmortem examination identified a clear cause of death in only 20% of the patients. Because most of the patients were not monitored continuously, it is difficult to differentiate patients who had problems that were suddenly recognized from those who had problems that occurred suddenly. The incidence of mortality did not change during the study period (after the first 3 days). Many factors may have influenced the mortality rate: change in intraoperative and postoperative care, cancer stage, and severity of coexisting diseases. In the case control study, patients were matched on age (±5 years), because the mortality rate is more closely related to medical history than to age.[7] The match on sex is justified by the high prevalence of upper airway cancers in men. Case control patients were initially chosen over the 3 months before surgery to eliminate a possible modification in the behavior of anesthetists or surgeons after early patient death. Nevertheless, because of the difficulty in finding control patients and maintaining the match on age and sex, the interval to surgery was widened to 1 year for 6 control patients.

The causes of death in our study have already been reported in several studies of postoperative complications after laryngectomy.[3,7,8] Myocardial infarction, cerebral stroke, and COPD exacerbation are the most frequent complications following neck dissection. The mortality incidence found in our study was

TABLE 4. POSTOPERATIVE PARAMETERS FOR DECEASED AND CASE CONTROL PATIENTS

	Deceased	*Control*
Postoperative potassium level (mmol/L)	4.2 ± 0.5 (12)	4 ± 0.4 (39)
Postoperative urea (mmol/L)	4.5 ± 2.2 (12)	4.3 ± 2.3 (39)
Postoperative creatinine (μmol/L)	78 ± 33 (12)	78 ± 25 (39)
Normal postoperative electrocardiogram (Y/N)	2/3	13/8
Postoperative P-R interval (s)	0.16 ± 0.04 (4)	0.16 ± 0.03 (20)
Postoperative Q-Tc interval (s)	0.48 ± 0.09 (4)	0.44 ± 0.05 (20)
Postoperative anticoagulation (Y/N)	1/10	10/35
Postoperative opiates (Y/N)	5/9	17/28

Data are mean ± SD, or numbers of patients. Numbers in parentheses are numbers of patients contributing to mean. No differences were significant.

similar to incidences previously reported.[3,4] Many situations may involve COPD exacerbation: pneumothorax (1% after total laryngectomy[8]), phrenic nerve section (0.25% to 1.45%[3,8,9]; 0% in our study), respiratory infection, and inhalation and asthma crises (1 death in 197 for Lunn et al[10] and 1 death in our study). Several mechanisms may induce postoperative upper airway obstruction: cervical hematoma (2.1% to 3.8%[11,12]), cervical edema, and obstruction of the tracheotomy cannula. Intracranial hypertension has not been reported in recent studies of postoperative mortality data,[3,4,6,8] but 1 patient died on day 7 of intracranial hypertension (0.02%) in our study. Intracranial hypertension may complicate bilateral ligature or unilateral ligature of the internal jugular vein, especially in patients with a contralateral jugular thrombosis (13% to 30% after functional neck dissection[13-15]). One of the most remarkable results is the very low incidence of death related to pulmonary embolism (2 of 3,015 patients) — much lower than that found after abdominal or gynecologic cancer surgery.[16] The incidence of pulmonary embolism was 0.01% in a study based on postmortem examination in a general hospital.[17] Lack of postoperative lower limb compression and early ambulation may have played a role in prevention of venous thrombosis. The incidence of death in relation to surgical complications was much lower than that previously reported[3,6,8]: 8 deaths (0.27%) during the 30 postoperative days in our study (tracheal and cervical bleeding). The lower rate of surgical complication–related deaths may be explained by the high degree of specialization of our surgical team.

The relationship between alcoholism and postoperative deaths is probably related to hepatic cirrhosis, although the findings on preoperative analysis were often normal. Chronic liver disease may produce little alteration in liver tests and may be clinically and biochemically undetectable. The hepatic reserve, however, may be limited, and decompensation may occur when an additional insult is introduced. Patients with compensated cirrhosis may be completely asymptomatic or have nonspecific symptoms. Patients with unsuspected liver disease who undergo exploratory laparotomy have a 31% mortality rate.[18] A Q-T interval prolongation occurs in some patients with alcoholic liver disease and is associated with an adverse prognosis, especially sudden cardiac death.[19] A close correlation has been noted between Q-T interval prolongation and vagal neuropathy in alcohol-related liver disease.[20] In our study, the mean Q-Tc intervals were the same in patients who died and those who did not (0.480 versus 0.440 second), as in the study of Day et al[19] (0.492 versus 0.463 second for men and 0.505 versus 0.480 second for women). In our study, the threshold of significance was not reached because of the low number of postoperative EKG examinations in the group of patients who died. Even in asymptomatic patients, careful evaluation should be performed to identify complications of alcoholism (hepatic, metabolic, or cardiovascular). A Q-T interval measurement should be included in the assessment of alcoholic patients.

Sudden death after neck dissection has occasionally been reported. It is often related to respiratory depression[21] or to cardiac complications.[22] A decreased ventilatory response to hypoxia has been reported after bilateral neck dissection.[23] Several medications, such as opioids, may induce severe respiratory depression after cervical dissection[21] by a decreased sensitivity of peripheral receptors; nevertheless, postoperative morphine administration was not found to be a factor predictive of death in our study. Otteni et al[22] studied Q-Tc interval modifications after radical neck dissection. In the postoperative period, they found that right radical neck dissection was followed by a Q-Tc interval prolongation that lasted until the second postoperative month. In their study, 2 patients had episodes of torsades de pointes and needed external cardiac massage (one at 10 hours and the other at 20 hours); a third patient died suddenly on day 10 without diagnosis. The invoked mechanism was a right cardiac sympathetic section.[22] In one study, it was shown that no significant change in the Q-Tc interval occurred after any neck dissection.[24] The number of patients included in that study was small, and the authors concluded that surgeons should be aware of the possibility of postoperative Q-Tc interval prolongation following neck dissection. In our study, there were more patients with a nerve section in the group of unexplained deaths than in the control group. The 3 patients who had a nerve X section died of an unexplained cause. (Two of them had a postmortem examination that did not find the cause of death.) It seems likely that cardiac dysrhythmia may represent a cause of sudden death in these patients.

One would expect that careful monitoring and follow-up would decrease the time between the beginning of the event and the arrival of a nurse. The observation and treatment of these patients must be improved by grouping the patients in the same ward (intensive care unit). Because of cost, it seems difficult to propose continuous monitoring of all patients by pulse oximetry and EKG for 3 days. Nevertheless, this monitoring may be applied to patients with certain risk factors: nerve section, alcoholism, and perhaps a history of cardiovascular or respiratory disease.

CONCLUSION

After neck dissection, there was a peak in the mortality rate during the first 3 days in relation to a peak in the incidence of sudden death. The exact mechanism of sudden death often remained unclear. In order to shorten the delay of treatment, careful follow-up of these patients in an intensive care unit seems indicated. Further studies on cardiovascular regulation and control of breathing are required to determine the mechanism of these sudden deaths.

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UNLOADED SHORTENING VELOCITY AND MYOSIN HEAVY CHAIN VARIATIONS IN HUMAN LARYNGEAL MUSCLE FIBERS

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Myosin description in human laryngeal muscles is incomplete, but evidence suggests the presence of type I, IIA, IIX, and tonic myosin heavy chain (MHC) fibers. This study describes the unloaded shortening velocity (Vo) of chemically skinned laryngeal muscle fibers measured by the slack test method in relation to MHC content. Skeletal fibers from human laryngeal and limb muscle biopsy specimens were obtained for determination of Vo, and subsequently, glycerol–sodium dodecyl sulfate–polyacrylamide gel electrophoresis was used to determine the MHC isoform content. The fibers from human limb muscle had shortening speeds similar to those in previous reports on human skeletal fibers. Type I, IIA, and IIX fibers of laryngeal muscle had shortening speeds similar to those of fibers from limb muscle, but laryngeal fibers with heterogeneous MHC expression had a wide range of shortening speeds, some being nearly twice as fast as limb fibers. In addition, MHC isoform bands from human extraocular muscle comigrated with some bands from laryngeal muscle — a finding suggesting that extraocular myosin may also be expressed.

KEY WORDS — laryngeal muscle fibers, myosin heavy chain, unloaded shortening velocity.

INTRODUCTION

The shortening velocity of mammalian skeletal muscle fibers is principally determined by variations in isoforms of the protein myosin. Myosin is a hexomeric molecule that has 3 basic functions: adenosine triphosphatase (ATPase) enzymatic activity, binding to actin, and sarcomeric thick filament formation. The speed of fiber shortening is correlated to enzymatic activity of the thick filament,[1] and myofibrillar ATPase activity is isolated to the myosin heavy chain (MHC) portion of the myosin molecule.[2] As such, there are many reports that positively correlate the type and amount of MHC isoform content to cellular shortening speed. The MHC isoforms typically found in somatic skeletal muscle include types I, IIA, IIB, and IIX. The relative shortening speeds of fibers that homogeneously express 1 isoform are IIB > IIX > IIA > I. However, when tested in various muscles and species, this structure-function correlation is often variable, especially where type II isoforms are concerned. Also, MHC isoform coexpression in somatic muscle fibers is relatively common,[3] with isoforms usually seen in combinations of I-IIA, IIA-IIX, and IIX-IIB. The contractile velocity in coexpressing fibers may span the entire range of both isoforms, such that correlation of shortening speed to the type and amount of MHC isoforms is further complicated. Some cranial muscles express novel MHC isoforms, which correlate to distinctive shortening speeds relative to somatic fibers — so much so that in theory these myosins evolved to serve highly specialized locomotor functions.[4,5] The jaw-closing muscles of all carnivores and primates investigated to date (except the lesser panda and human) express a type II masticatory MHC (IIM) that has a twitch contraction speed faster than that of fast limb muscles. Extraocular muscles are even faster than chewing muscles, with twitch contraction speeds as fast as about 7.5 ms.[6] Mammalian extraocular muscles also have a unique MHC termed extraocular myosin (EOM). Unlike chewing muscle fibers, which usually homogeneously express type IIM to achieve distinctively fast contraction, extraocular fibers coexpress EOM with other isoforms.

One of the last cranial muscle groups to be investigated for structural-functional relationships of myosin were the intrinsic laryngeal muscles. DelGaudio et al[7] were the first to report an "atypical" MHC isoform expressed in rat posterior cricoarytenoid (PCA) and thyroarytenoid (TA) muscles that was identified by a distinctive migration pattern on glycerol–sodium dodecyl sulfate–polyacrylamide gel electrophore-

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sis (glycerol-SDS-PAGE). This isoform was termed "type IIL" when present in laryngeal muscle, even though it comigrated with the EOM species from rat lateral rectus muscle. Lucas et al[8] subsequently demonstrated the presence of EOM expression in rabbit TA muscle by tissue staining with monoclonal antibody specific for EOM. Preliminary evidence also suggested that transcripts from a novel MHC gene were expressed in rat TA muscle,[9] but further study determined that these transcripts were from the EOM gene, and not a novel laryngeal myosin.[10] Finally, electrophoretic analyses on dogs[11] and humans[12] were unable to identify additional MHC species comparable to "IIL" or EOM MHC. Two reports, however, leave open the possibility that atypical or novel MHC isoforms may exist in human intrinsic laryngeal muscles. Shiotani et al[13] have identified typical MHCs I, IIA, and IIB by electrophoresis in human laryngeal muscle and, in addition, a protein band with mobility similar to that of the rat IIL reported by DelGaudio et al that does not react with EOM antibodies. A subsequent report found a similar band on glycerol gels in TA and PCA muscles of a 7-month-old infant, but adults 40 years and older did not contain this protein species.[14]

In whole muscle preparations, evaluation of isometric twitch contraction speed demonstrates shortening for TA muscle as fast as 6.5 to 14 ms in rabbits,[15] dogs,[16] primates,[17] and cats.[18] In cats[18] and dogs,[16] isometric contraction speeds in PCA muscles range from 22 to 40 ms, which are comparable to speeds of fast-contracting limb muscles such as the rabbit tibialis anterior (24 to 28.5 ms)[15] and the cat extensor digitorum longus (40 ms).[6] Because whole muscle preparations that are comparable to isometric contraction experiments in animals are impossible to perform on humans, investigators have relied upon indirect assessment of contraction speeds. A study of changes in human vocal fold vibration after electrical stimulation of laryngeal muscle estimated TA contraction speed to be about 35 ms.[19] More recently, Luschei et al[20] attempted to determine the speed of laryngeal muscle motor unit recruitment by measuring interspike interval variability during phonation. According to their data, the contraction speed of human laryngeal muscle is not faster than that of limb muscles such as the tibialis anterior or biceps. An immunohistochemical analysis has determined that a tonic MHC isoform is expressed in human TA muscle fibers,[21] indicating that these fibers should be optimally suited for very slow contraction speeds. Collectively, these recent findings from humans estimate that the shortening speeds in laryngeal muscle are about the same as or even slower than limb muscles and, hence, very different from the findings of direct work on other mammalian larynges.

In summary, investigations to determine structure-function relationships of myosin in human laryngeal muscles are lacking. Further, most of the recent evidence on human laryngeal muscle suggests that their shortening speeds are not comparable to those of other mammals. Accordingly, the present study attempts to determine the relationship between myosin isoform content and unloaded shortening velocity (Vo) in single fibers from human laryngeal muscles.

MATERIALS AND METHODS

Materials. Samples of TA and PCA muscles were obtained from 4 patients who underwent laryngectomy as a treatment for cancer. Presurgical diagnostic laryngeal evaluation by flexible laryngoscopy was done to ensure that normal neuromotor laryngeal function (ie, normal breathing, swallowing, and phonation) was present in these subjects. No individuals with vocal fold paralysis were included. Excision of 3 TA muscles and 2 PCA muscles from uninvolved areas of the larynx was performed immediately after laryngectomy and before pathology submission. The subject population was of mixed race and sex and had an average age of 67 years (range, 50 to 78 years). Two control muscle biopsy samples were obtained from vastus muscles (24-year-old woman and 46-year-old man), 1 sample was obtained from a tibialis muscle (33-year-old man), and 1 sample was obtained from an external oblique muscle (57-year-old woman). The control muscles were functionally normal and were obtained from a population being treated by an orthopedic oncology surgeon for removal of a tumor that did not involve the sampled muscle. The samples for this study were obtained according to the guidelines established by the university's Internal Review Board for Human Subjects.

Biochemistry. The samples of whole muscle were minced and then homogenized in buffer containing 0.1 mol/L potassium chloride, 5 mmol/L ethylenediaminetetraacetic acid (EDTA), 5 mmol/L ethyleneglycoltetraacetic acid (EGTA), and 1 mmol/L dithiothreitol, pH 7.0, and the contractile proteins were sedimented by centrifugation.[22] The final pellet was resuspended in electrophoresis sample buffer (0.15 mol/L Tris–hydrochloric acid [HCl], 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.002% bromophenol blue, pH 6.8). These protein samples were vortexed, heated to 100°C for 5 minutes, and then stored at –70°C until electrophoresis was carried out. Individual segments of single fibers that were used for Vo measurements were placed in sample buffer, ultrasonicated for 15 minutes, and then heated and frozen.

Discontinuous glycerol-SDS-PAGE was conducted on 0.75-mm-thick separating gels of 9% (wt/vol) acrylamide cross-linked with bisacrylamide (200:1.0, acrylamide:bisacrylamide), 10% SDS, and 6.0% glycerol, buffered with 0.75 mol/L Tris-HCl at pH 8.8. The stacking gel contained 4% acrylamide cross-linked with bisacrylamide (20:1.0, acrylamide:bisacrylamide) with 0.5 mol/L Tris buffer at pH 6.8. The running buffer consisted of 0.3% Tris-HCl, 1.44% glycine, and 0.1% SDS. The upper electrode running buffer also contained 0.1% β-mercaptoethanol. Proteins were electrophoresed at 4°C with a constant current of 25 mA for about 2 hours to allow the samples to pass from the stacking gel into the separating gel. The current was increased to 40 mA, and electrophoresis continued until 8,000 volt-hours (approximately 22 hours of total running time). The gels were stained with silver according to the method of Merril et al.[23] Densitometric analyses were done on the stained gels in an AGFA DuoScan with Quantity 1 analysis software (BioRad Corp, Hercules, California).

Immunoblotting. Protein extracts from chemically skinned fibers or muscle homogenates placed in sample buffer as described above were applied to nitrocellulose membrane under a gentle vacuum with a Hoeffer PR600 slot blot apparatus. The blotted membranes were blocked with powdered milk in phosphate-buffered saline solution and subsequently incubated in the primary anti-MHC antibodies BA-F8 (anti–type I), SC-71 (anti–type IIA), BF-F3 (anti–type IIB), and MY-32 (anti-type II). The antibody reactivity in the fibers was detected after incubation in secondary antibodies by the indirect immunoperoxidase method.[5]

Physiology. Portions of the muscle samples were microdissected into fiber bundles 2 to 3 mm in diameter and placed into a pre-skinning solution. This solution was prepared by adding 0.1 mmol/L phenylmethanesulfonyl fluoride (a serine protease inhibitor) to a relaxing solution (see below). The bundles were gently agitated for 4 to 6 hours, during which the pre-skinning solution was changed 2 or 3 times until free from discoloration. The bundles were then placed in a 50/50 pre-skinning–glycerol solution and gently agitated for approximately 12 hours at 4°C before being stored in the same solution at −30°C for up to 2 months. On the day of the experiment, single fibers were isolated in relaxing solution by pulling from one end of the bundle. The relaxing solution contained (mmol/L): 20 N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (Bes), 7 potassium-EGTA, 4 sodium–adenosine triphosphate, 14.5 creatine phosphate, 1 free magnesium ion, and sufficient potassium propionate to adjust the ionic strength to 180 mmol/L. Propionate was the major anion in all solutions. The free calcium ion concentrations were 10^{-9} for the relaxing solution and 10^{-4} for the activating solution, respectively. Free bivalent cation and metal-ligand complex concentrations were estimated with the computer program "Winmaxc,"[24] obtained from URL http://www.stanford.edu/~cpatton/maxc.html. Single fibers (1.5 to 5 mm long) were mounted via small stainless steel clamps between a force transducer and the linear motor of the physiological test rig (Scientific Instruments, Heidelberg, Germany) in relaxing solution. The test rig was interfaced to a personal computer that controlled the experimental protocol–acquired data via dedicated software (SKI, Scientific Instruments). All experiments were performed at 15°C. A 670-nm helium-lanthanum laser beam was directed onto the fiber, and the resulting diffraction pattern was focused onto a ground glass screen. The initial sarcomere length was adjusted to approximately 2.5 µm, as calculated from the first deflection distance of the diffraction pattern produced. Before activation, the relaxing solution was exchanged for a pre-activating solution, with reduced calcium ion–EGTA buffering capacity, for 20 to 30 seconds. The pre-activating solution was identical to the relaxing solution, except that the potassium-EGTA was reduced to 0.5 mmol/L; this reduction resulted in a faster rise to steady tension after activation and improved the maintenance of cross-striation uniformity during activation.[25] The fiber was then induced to contract in a maximal activating solution (pCa 4.0) until a stable force was recorded. A slack test was then conducted to determine the maximum velocity of unloaded shortening of skinned fibers (also termed V0).[26] For the slack test, the fiber was allowed to shorten by a series of 5 incremental length changes, typically 10% to 15% of resting length for up to 3.0 seconds' duration, after which resting length was restored. For each length change (ΔL), the time it took the fiber to shorten and just redevelop force (ΔT) was measured. The graph of ΔL versus ΔT was fitted with a straight line by least squares regression, and V0 was taken as the slope of this line (Fig 1). Data with an r^2 value greater than 0.98 were rejected.

RESULTS

Electrophoresis. Using whole muscle and single fiber samples from external oblique and vastus muscle biopsy specimens, we were able to establish MHC control standards for type I, IIA, and IIX isoforms (Fig 2). The fastest-migrating band on gels stained positively for the type I antibody (BA-F8), but was negative for all of the fast antibodies on slot blots.

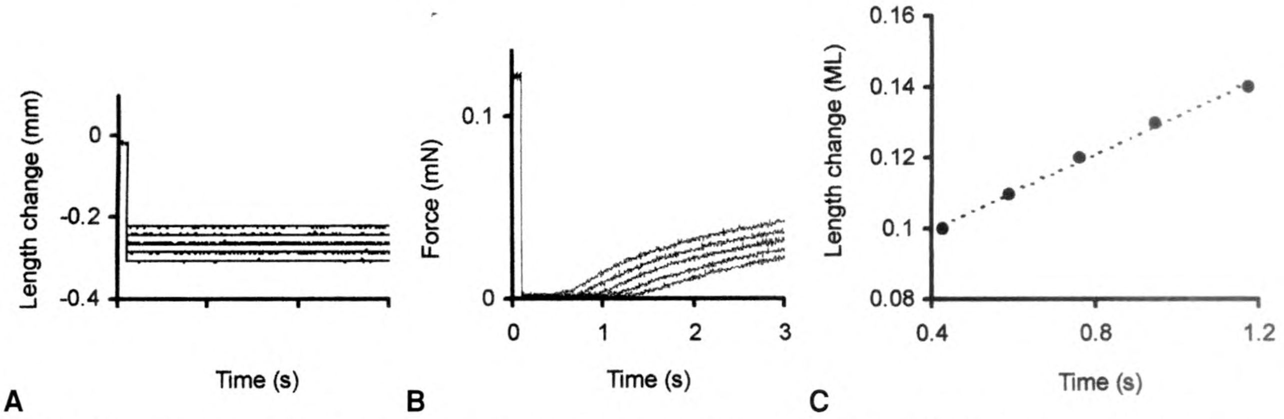

Fig 1. Determination of unloaded shortening velocity (V0) in skinned skeletal muscle fibers by slack test protocol. **A**) During maximal activation of fiber, series of step reductions in length were performed (superimposed). **B**) Shortening times necessary for fiber to remove slack were measured (superimposed). mN — millinewtons. **C**) Slope of relationship between length change and shortening time determined V0 in muscle lengths (ML) per second.

The intermediately migrating band on gels stained positively for the type IIA antibody (SC-71) and the general fast antibody (MY-32), but not the IIB antibody (BF-F3). The slowest-migrating band stained positively only for the general fast antibody. Thus, electrophoretic standards whose relative mobilities correspond to protein species I > IIA > IIX were then used to identify MHC isoforms from whole muscle and single fiber preparations in the TA and PCA muscles. Whole muscle TA and PCA samples contained variable amounts and types of MHC isoforms. Some biopsy samples contained only variable amounts of the typical isoforms I, IIA, and IIX, but others contained 2 additional fast-migrating bands (Fig 3). Given evidence from animal studies of additional myosin isoforms present in some species, we also investigated the pattern of isoform banding from human inferior oblique (extraocular) muscle, fetal muscle, and atrium (cardiac) muscle. The inferior oblique muscle contained type I, IIA, and IIX MHCs, but 3 additional fast-migrating bands were also present. The band with a migration speed slightly faster than that of type I comigrated with the neonatal MHC from fetal muscle. The additional 2 bands comigrated with the additional bands seen in laryngeal muscle. The α-MHC band from the atrial sample migrated between the type IIX and IIA bands, and was not detected in any of the skeletal muscle samples.

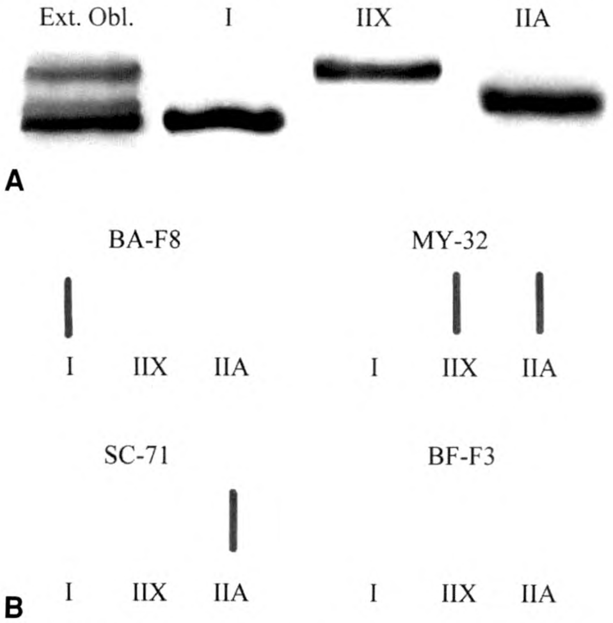

Fig 2. Identification of myosin heavy chain isoforms present in human limb muscle, from whole muscle biopsy homogenates and single fibers. **A**) Mobilities of proteins from biopsy homogenate of external oblique (lane 1) corresponded to type I, IIA, and IIX isoforms from single fibers that expressed only 1 MHC (lanes 2, 3, and 4). **B**) Identification of MHC isoforms from standard single fibers were confirmed in immunologic slot blots by means of BA-F8 (anti–type I), MY-32 (general anti-fast), SC-71 (anti–type IIA), and BF-F3 (anti–type IIB) antibodies. No antigenic activity for IIB antibodies was detected in single fibers.

Physiology. We determined both the V0 (using the slack-test method) and the MHC isoform content (using glycerol-SDS-PAGE) for 38 control fibers, 28 PCA fibers, and 29 TA fibers. The vastus, tibialis, and external oblique fiber physiology results were grouped together as control data to determine whether the results of our techniques were similar to those of previous reports of V0 in human skeletal muscle. The V0 values for fibers that homogeneously expressed MHC in control muscles were (in muscle lengths per second) type I, 0.508 ± 0.089 (mean ± SEM, n = 18); type IIA, 0.783 ± 0.072 (n = 10); and type IIX, 2.218 (n = 1; see Table and Fig 4). Fibers with heterogeneous myosin expression were grouped together, producing a V0 value of 0.726 ± 0.127 (n = 9). These results were comparable in 2 respects to those of previous reports on human skeletal muscles.[25] The V0 generally increased in homogeneous fibers in the order I, IIA, IIX. Second, the speed of shortening in

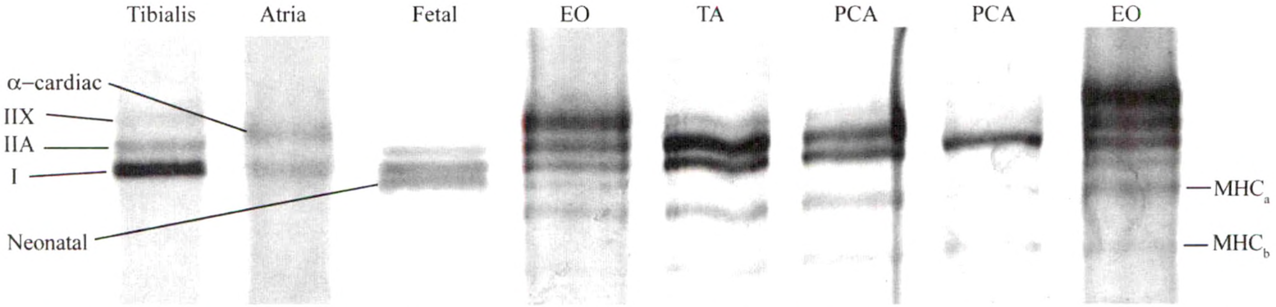

Fig 3. Glycerol–sodium dodecyl sulfate–polyacrylamide gel electrophoresis of whole muscle biopsy homogenates of human tibialis, atria, fetal, extraocular (EO), thyroarytenoid (TA), and posterior cricoarytenoid (PCA) muscles. Relative mobilities of known protein species were neonatal > I > IIA > α-cardiac > IIX. Two additional bands (labeled MHC$_a$ and MHC$_b$) were always present in EO muscle and sometimes present in TA and PCA muscles.

hybrid fibers correlated with the type and amount of MHC as determined by glycerol-SDS-PAGE and densitometry. In the PCA muscle, type I fibers had V_0 values of 0.491 ± 0.056 (n = 23), and type IIA, 1.255 (n = 2). Fibers with heterogeneous myosin expression in the PCA muscle had a V_0 value of 2.898 ± 1.45 (n = 3). In the TA muscle, the V_0 values for fibers that homogeneously expressed MHC were type I, 0.787 ± 0.115 (n = 3); type IIA, 0.851 ± 0.39 (n = 6); and type IIX, 1.36 ± 0.729 (n = 3). Fibers heterogeneous for MHC expression had a V_0 value of 1.140 ± 0.28 (n = 17; Fig 4).

DISCUSSION

In this study, we present preliminary evidence that the V_0 in single chemically skinned fibers from human PCA and TA muscles spans a wide range of speeds. Type I, IIA, and IIX laryngeal fibers were comparable in V_0 to control fibers from human limb muscle. Some of the laryngeal fibers, however, shortened nearly twice as rapidly as their counterparts from fast-contracting limb muscle. The fastest-contracting fibers from laryngeal muscle always had heterogeneous MHC expression. To our knowledge, this is the first examination of the physiological properties of individual skeletal fibers dissected from human laryngeal muscles.

Myosin Expression in Human Laryngeal Muscle. Ten MHC isoforms have been identified in mammalian muscle, which may be subdivided into the following groups: 1) developmental isoforms (embryonic and neonatal); 2) slow isoforms (I or slow twitch contraction, and tonic or slow tonic contraction); 3) fast isoforms (IIA, IIB, and IIX); 4) very fast cranial isoforms (IIM in masticatory muscles and EOM in extraocular and laryngeal muscles); and 5) cardiac isoforms (α and β; β is the same isoform as type I in skeletal muscle; for a general review, see Schiaffino and Reggiani[3]). All of these isoforms have been identified in human skeletal muscle, with the exception of type IIB. Fibers formally classified as IIB with myofibrillar ATPase histochemistry have more recently been determined to contain a homolog of the type IIX protein isoform, rather than IIB.[27] Although IIB protein has not as yet been positively identified in human muscle, the MHC-IIB gene has been sequenced[28] — a finding suggesting that a gene message may be present.

FIBER UNLOADED SHORTENING VELOCITY

Fiber Type	*Control Muscle*	*PCA Muscle*	*TA Muscle*
I	0.508 ± 0.089 (18)	0.491 ± 0.056 (23)	0.787 ± 0.115 (3)
IIA	0.783 ± 0.072 (10)	1.255 (2)	0.851 ± 0.39 (6)
IIX	2.218 (1)		1.360 ± 0.729 (3)
Heterogeneous	0.726 ± 0.127 (9)	2.898 ± 1.45 (3)	1.140 ± 0.28 (17)

Data are mean ± SEM (n in parentheses).
PCA — posterior cricoarytenoid, TA — thyroarytenoid.

The first reports on myosin expression in human laryngeal muscle were based on histochemical fiber typing, which described differences in type I versus type II fiber populations. Most of these reports concluded that TA muscle contains the most fast fibers (about 65%) and that the PCA muscle contains roughly equivalent proportions of type I and type II fibers.[29,30] A recent histochemical report confirms these earlier results on human TA muscle.[31] Immunohistochemical identification of myosin isoforms has only recently been investigated, and shows that antibodies reactive for type I MHC, general fast type II MHCs,[32] tonic MHCs,[21] and neonatal MHC do react with fibers in human TA muscles. The neonatal MHC is thought to be expressed in regenerating fibers associated with age-related muscle changes,[33] rather than as part of normal protein expression. A comprehensive immunohistochemical and histochemical fiber type classification scheme on TA and other human laryngeal muscles has yet to be developed.

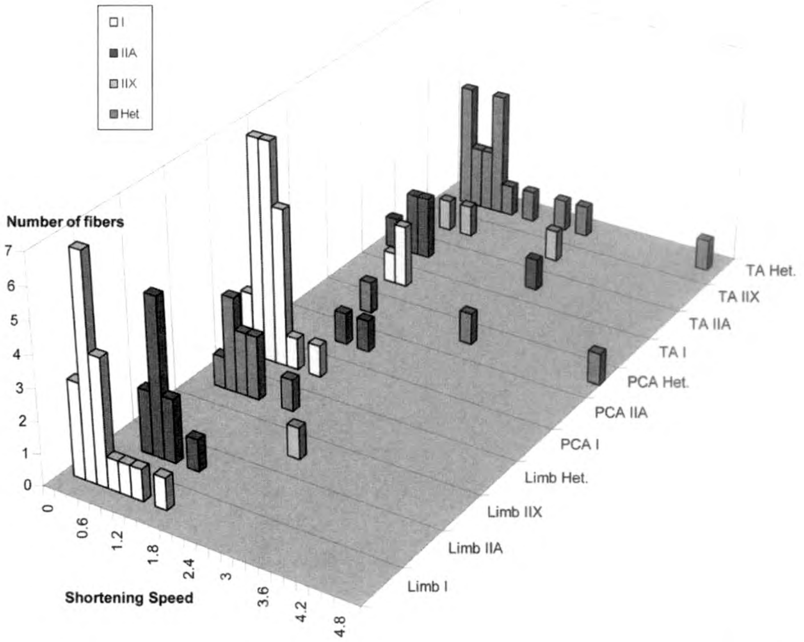

Fig 4. Shortening speed assessed by grouping fibers according to muscle and fiber type. Het. — heterogeneous.

Biochemical isolation of MHC isoforms by electrophoresis is made possible by modifying the SDS-PAGE technique. By variation of the proportions of acrylamide and bisacrylamide and addition of glycerol to the gel, isoforms of myosin were shown to be separated by properties other than molecular weight.[34] Although not yet directly tested, this technique most likely relies on variations in post-transcriptional modifications such as protein glycosylation. Since this separation is not based on protein weight, MHC migration speeds relative to each other sometimes vary by species rather than by isoform. For example, in rats, IIA and IIX isoforms do not always separate from each other,[7] but in humans, IIA and IIX isoforms are consistently resolved as distinct bands.[22] Hence, it may not always be possible to determine isoform identity by comparing the myosin banding pattern in one species to that in another. In rats, EOM can be separated from other fast MHC isoforms in the extrinsic eye muscles and laryngeal muscles.[7] In addition to this present study, 3 reports have described MHC banding by glycerol-SDS-PAGE in humans. One of these reports identifies only type I, IIA, and IIX isoforms.[12] The 2 other reports have identified an additional myosin band that has yet to be positively identified.[13,14]

The samples from human PCA and TA muscles analyzed here contained either 1 or 2 additional MHC bands that, in comparison to whole muscle homogenates of limb control muscle samples, were not type I, IIA, or IIX isoforms. Homogenates from human atrium, known to contain α MHC, and from fetal muscle, known to contain neonatal MHC, were also investigated to determine whether the additional bands in laryngeal muscle were these isoforms. The α isoform consistently migrated intermediately between the IIA and IIX bands, and the neonatal isoform migrated slightly faster than the type I band. The additional protein species in human laryngeal muscle did not comigrate with α or neonatal MHC. Since extraocular muscle is thought to express at least 6 MHC genes,[35] samples of human inferior oblique muscle were obtained. The extraocular muscle contained types I, IIA, and IIX, as found in control limb muscle, and neonatal MHC, as found in fetal muscle. Two additional bands in extraocular muscle comigrated with the additional bands seen in laryngeal muscle. From our control data, we excluded type IIX, α, IIA, I, and neonatal MHCs, and suggest that one of these additional bands is human EOM and the other is either tonic or embryonic MHC.

V0 and MHC Composition. The shortening speeds of limb muscle fibers were obtained to compare the results of our slack test protocol to those previously reported for human muscle. In a study that included relatively large numbers of soleus and quadriceps fibers, type I, IIA, and IIX fibers had respective shortening speeds of 0.3, 1.0, and 3.1 muscle lengths per second.[25] The range of speeds for these fibers was inclusive of the mean data of our results for I, IIA, and IIX fibers (0.508, 0.783, and 2.218 muscle lengths per second, respectively) from human vastus, triceps, and external oblique muscles. Also, as reported previously, the shortening speed of chemically skinned fibers correlated to the type of MHC isoform present. Type I, IIA, and IIX fibers from TA and PCA muscles also had shortening speeds within the anticipated range for human limb fibers. Some of the fibers with heterogeneous expression of MHCs, however, had unusually fast contraction times, approaching twice the speed of fast-contracting type II fibers. These fibers were identified in both the TA and PCA muscles sampled. Gels from these fibers showed only type I, IIA, and IIX MHCs, and not the additional bands found in whole muscle homogenates of PCA and TA muscles. Why some fibers that heterogeneously express MHCs from TA and PCA muscles have unusually fast shortening speeds remains to be determined. Extraocular muscle fibers with comparably fast contraction speeds are known to coexpress EOM along with other isoforms. Our heterogeneous fibers may have small amounts of EOM that are beyond the limits of detection with the glycerol-SDS-PAGE technique. Another possible explanation for fast shortening speeds may be that factors other than MHC composition affect these properties. Further descriptions of cellular kinetics in relation to the type and amount of contractile and regulatory proteins are necessary in order for us to understand how physiological activity is determined in laryngeal fibers. Ultimately, direct assessment of cellular activity should be compared to indirect clinical assessments of human laryngeal muscle function, to elucidate further the functioning of this organ. The findings in this study of diverse laryngeal muscle fiber types (and varying contraction speed) could be explained by review of the muscle activities and functions of the larynx. Laryngeal functions involve voice, respiration, and swallowing. Respiratory activities of the larynx require periodic, frequent rapid firing of the PCA muscle to abduct the vocal folds. This muscle activity is probably quite similar to the TA muscle activities during initiation and variation of speech and singing (rapid muscle contraction). However, laryngeal muscle activities also require constant and prolonged muscle contraction, notably during sustained phonation (both TA and PCA muscles).

Preliminary investigations of the individual laryngeal muscles and their activities have found that there are different subgroups of laryngeal muscles with unique functions. This specialization is most recognized in the PCA muscle. There are 2 bellies of the PCA muscle: horizontal and vertical. The former appears to be involved in vocal fold abduction for respiration, and the latter in maintenance of vocal fold tone, bulk, and possibly lengthening. There have been similar anatomic and functional subcompartments proposed for the TA muscle.[36] Given the disparate findings of the muscle fiber types and properties found in this study, we hypothesize that our random muscle sampling involved different areas of the laryngeal muscles under investigation. Future studies are planned that would use a reliable method of muscle harvesting from similar subregions of the TA and PCA muscles.

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WAVEFORM CHANGES IN ANTIDROMIC FACIAL NERVE RESPONSES IN PATIENTS WITH BELL'S PALSY

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We repeatedly tested the antidromic facial nerve response within 7 days after onset of paralysis in patients with Bell's palsy. None of 109 patients showed the triphasic waveform that reflects normal conduction of the facial nerve action potential. The waves recorded from patients showed biphasic, monophasic, or flat waveforms. Eighty-two of 88 patients with complete recovery showed biphasic waves, whereas half of the patients with nerve degeneration had monophasic or flat waves. Most patients with complete recovery maintained biphasic waves, but in patients with incomplete recovery, the waveforms changed to monophasic or flat, except in 1 case. The presence of monophasic or flat waves with a low facial score strongly suggests nerve degeneration. The antidromic facial nerve response is recommended as a method of diagnosing paralysis and monitoring the progression of intratemporal facial nerve damage during its early stages.

KEY WORDS — antidromic facial nerve response, Bell's palsy, facial nerve monitoring, nerve action potential.

The antidromic facial nerve response (AFNR) was originally reported by Bumm et al[1] in 1974 as a method of monitoring intratemporal facial nerve damage during the early stages of paralysis. However, the test has not gained widespread acceptance, because recording the tiny nerve response was very difficult in human subjects. One reason for the difficulty in recording a response is that the facial nerve was stimulated over the skin in previous studies.[2-5] The high electrical resistance of the skin and the subcutaneous tissue requires relatively large electrical currents, which produce enough large stimulation artifacts to disturb detection of the AFNR. Therefore, we introduced a method of stimulation via Stensen's duct.[6,7] This method greatly reduced the stimulating current and made it easy to identify the nerve response. Moreover, we further modified the recording technique and found it possible to record the AFNR clearly in outpatient clinics.[6-8]

According to Tashima et al,[9] who discussed the waveform of the AFNR in animal experiments, the normal AFNR showed a triphasic waveform composed of 1 negative peak and 2 positive peaks (Fig 1). However, in damaged nerves, the waveform was changed to biphasic, monophasic, or flat (Fig 2). This change was determined by the relationship between the nerve conduction–blocking site and the recording site. When the blocking site was more proximal than the recording site, the waveform was biphasic. If the blockade was located around the recording site, the waveform became monophasic, as shown in a so-called killed-end potential. If the blockade was peripheral to the recording site, the response was no longer detected. In other words, the abnormal waveforms indicated the existence and the site of the nerve conduction block.

Although advances in electrodiagnosis have made possible early detection of intratemporal facial nerve paralysis, the conventional tests, such as the nerve excitability test and electroneurography (ENoG), require more than 1 week to diagnose nerve degeneration.[10-13] We wish to detect nerve degeneration as soon as possible, because early detection is essential in selecting appropriate treatment of facial paraly-

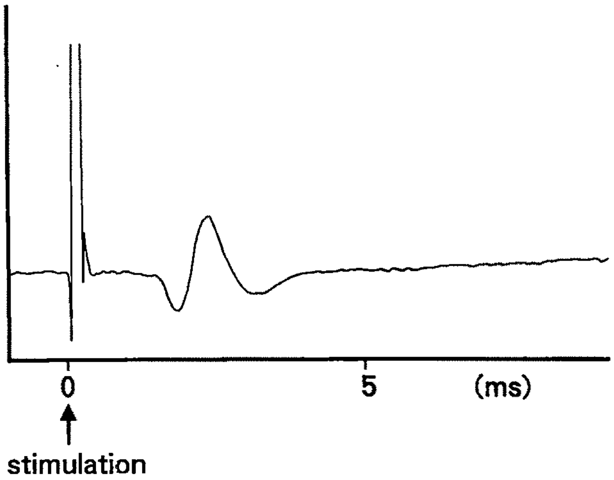

Fig 1. Normal waveform of antidromic facial nerve response (AFNR), which shows triphasic waveform composed of 1 negative peak and 2 positive peaks.

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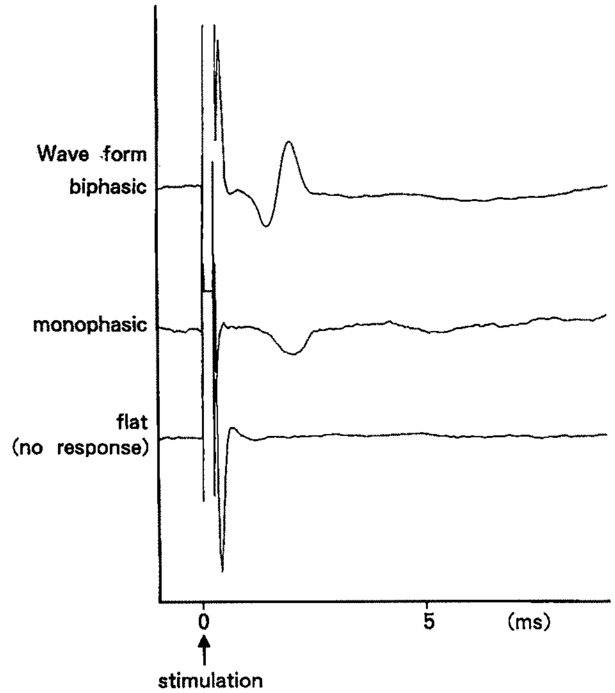

Fig 2. Abnormal waveforms of AFNR.

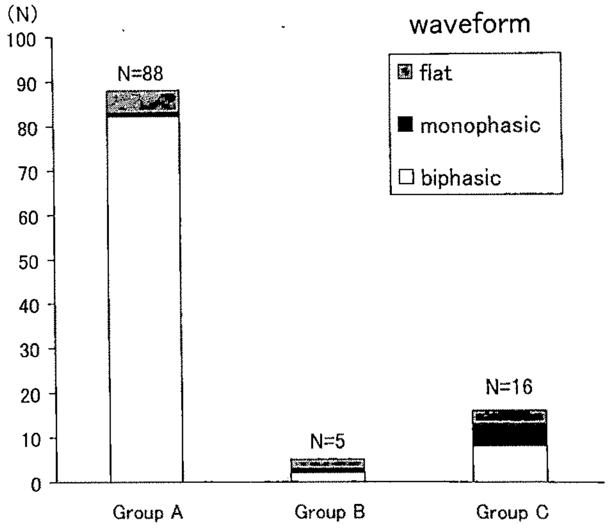

Fig 3. Waveforms at first recordings. Most patients showed biphasic waves, especially in group A. In groups B and C, half of patients had monophasic or flat waves.

sis. According to the study of Yanagihara et al,[14] the site of the initial lesion of Bell's palsy is related to the outcome of paralysis. Therefore, we detect nerve degeneration during the early stages from waveform analysis of the AFNR. The purpose of the present report is to describe waveform changes in AFNR in cases of early-stage paralysis and to discuss whether the AFNR is able to detect nerve degeneration within 1 week after the onset of paralysis.

MATERIALS AND METHODS

A total of 109 patients with Bell's palsy who visited our hospital within 7 days after the onset of paralysis were examined in the present study. All patients were treated conservatively and were followed up for at least 6 months to confirm the outcome of paralysis. Fifty-eight of the patients were male, and 51 were female. Their ages were between 11 and 83 years (mean ± SD, 47.2 ± 18.0 years). Before testing, all patients gave their consent to the study.

The recording methods used have been described previously.[6-8] The facial nerve was stimulated through Stensen's duct at a depth of 5 cm from the orifice, with a bipolar catheter electrode of 0.8- or 0.6-mm diameter (UKG100-2PM or UKG100-2PM-19G, Unique Medical, Tokyo, Japan). We set the stimulation intensity at a current 1 mA higher than the threshold of facial nerve excitation. After local anesthesia of the external auditory canal, the 2-channel active electrodes (a monopolar needle electrode, OH90-039, NEC Sanei, Tokyo) were placed at the posterosuperior part of the annulus tympanicus and at the entrance to the external auditory canal for the following subtraction method. A silver-plated surface electrode was placed at the forehead as the common reference electrode of both channels. Another surface electrode was placed on the mastoid process as the ground electrode. The nerve response was amplified with filter settings of 50 to 3,000 Hz, and 200 series of responses evoked by pairs of pulses with alternating polarity at a frequency of 10/s were averaged by a signal processor (MEB-7102, Nihon Koden, Tokyo). Then, the response evoked at the entrance to the external auditory canal was subtracted from that recorded at the annulus tympanicus, to eliminate the compound muscle action potential of the facial muscles and to detect only the nerve action potential.

In each patient, the AFNR was recorded within 7 days after the onset of paralysis. The severity of paralysis was evaluated by the 40-point Japanese grading system,[15] the House-Brackmann grading system,[16] and pathological synkinesis. Then, the patients were divided into 3 groups (A through C) according to severity. Group A had a facial score of at least 36 points and no synkinesis. Group B had a facial score of at least 36 points and mild synkinesis. Group C had a facial score of less than 36 points and severe synkinesis. The degree of nerve damage was considered to be no degeneration in group A (88 patients), partial degeneration in group B (5 patients), and total degeneration in group C (16 patients). On the House-Brackmann grading system, the outcomes of group A, B, and C were equivalent to grade I, grade II, and grade III or IV, respectively. In each group, the waveform changes of the AFNR were examined.

RESULTS

Figure 3 shows the waveforms obtained from the

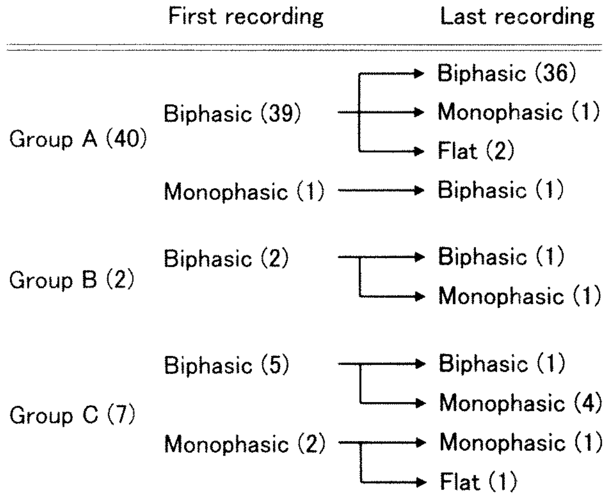

Fig 4. Changes in waveform. Most patients in group A maintained biphasic waves at final recording, but 6 of 7 patients in group C had monophasic or flat waves.

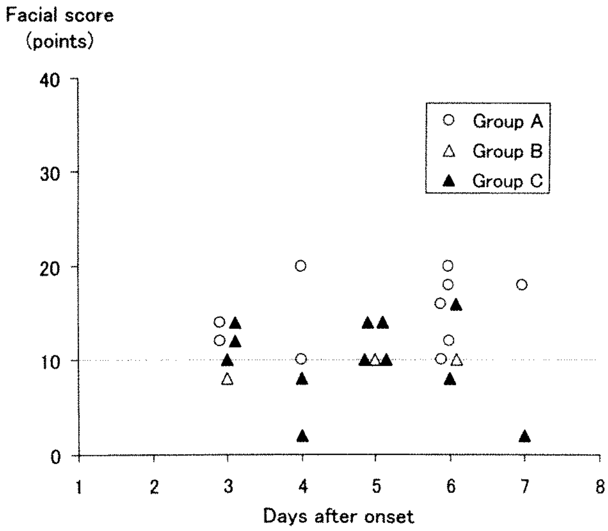

Fig 5. Facial scores[15] at time monophasic or flat waves were recorded. All patients in group A had scores of more than 10 points, whereas all 3 patients in group B and 7 of 12 patients in group C had scores of less than 10.

patients at the first recordings in each group. No patient showed a triphasic waveform. Ninety-two of the 109 patients (84.4%) had biphasic waves. In group A, 82 of the 88 patients (93.2%) had biphasic waves, and the other patients had a monophasic wave in 1 case and flat waves (no response) in 5 cases. In group B, the waveforms were biphasic in 2 patients, monophasic in 1 patient, and flat in 2 patients. In group C, the waveforms were biphasic in 8 patients, monophasic in 5, and flat in 3.

Forty-nine patients were repeatedly tested within 7 days after the onset of paralysis (Fig 4). In group A, 40 patients were tested more than twice in the first week of paralysis, and 39 patients (all except for the 1 patient with the monophasic waveform) had biphasic waves at the first test. During the course of subsequent tests, 36 retained their biphasic waves; however, the waveform changed to monophasic in 1 patient and flat in 2 patients. The patient who had the monophasic waveform at first showed a biphasic wave at the end of the test period. In group B, only 2 patients had repeated tests. Both patients showed biphasic waves at the first test; 1 patient maintained the biphasic wave, and the other showed a monophasic wave on the 6th day after the onset of paralysis. In group C, 7 patients were repeatedly tested; 4 of the 5 patients who had biphasic waves at first testing later showed monophasic waves, and the other patient maintained the biphasic waveform. Of the 2 patients who first had a monophasic waveform in group C, 1 patient maintained the monophasic wave, and the last test showed no response in the other patient.

Figure 5 shows the facial scores obtained on the Japanese grading system[15] at the time the monophasic or flat wave was recorded. In group A, 10 of 88 patients (11.4%) had these waves within the first week of paralysis, and all patients scored more than 10 points. Five patients were tested only once, and the waveform returned to biphasic in 2 of the other 5 patients. However, 3 of 5 patients (60%) in group B and 12 of 16 patients (75%) in group C showed monophasic waves. All 3 patients in group B and 7 of 12 patients in group C scored less than 10 points.

CASE REPORTS

Case 1. A 33-year-old woman had herpetic vesicles on her lower lip and severe headaches 3 days before her first visit. After 2 days, she noted left facial paralysis and was referred to our hospital after consultation with her family doctor. The otoscopic findings were normal, and no eruptions were observed in the auricle or the external auditory canal. Pure tone audiometry and vestibular function tests showed normal findings. There was no remarkable increase in the paired serum antibody titers of varicella-zoster virus (VZV) or herpes simplex type I virus (HSV-I). Her facial movement was scored 16/40 on the Japanese grading system[15] and grade III on the House-Brackmann system.[16] A stapedial reflex was obtained in the left ear, and the lacrimation of the left eye was 63% of that of the right eye. The ENoG revealed 35.2% degeneration on the left side, and the nerve excitability test (NET) was normal. The AFNR showed a biphasic wave, and its amplitude was 6.25 μV (Fig 6). Intravenous steroid therapy was started, and the facial score and NET, ENoG, and AFNR findings were investigated every day up to the 5th day after paralysis. The facial score and ENoG and NET findings did not worsen, and the AFNR maintained the biphasic waveform. The patient's facial movement improved 2 weeks later, and had almost recovered 1 month af-

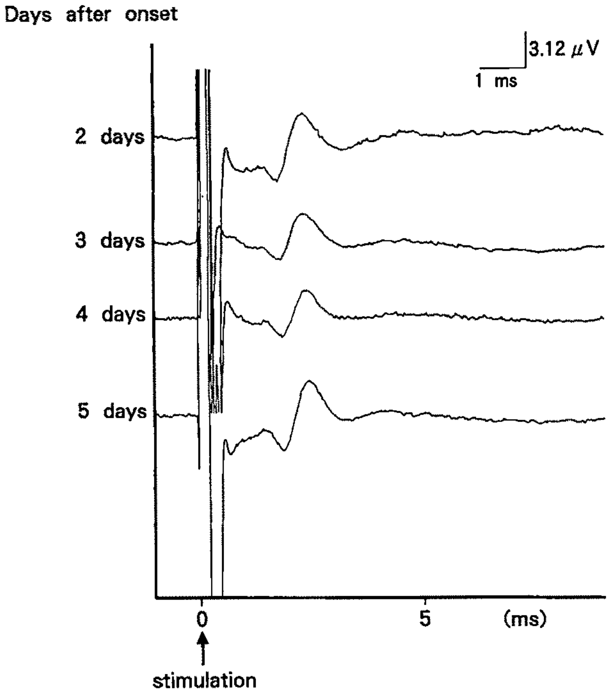

Fig 6. (Case 1) Waveform changes in patient with complete recovery. From 2 days to 5 days after onset of paralysis, patient maintained biphasic waveform.

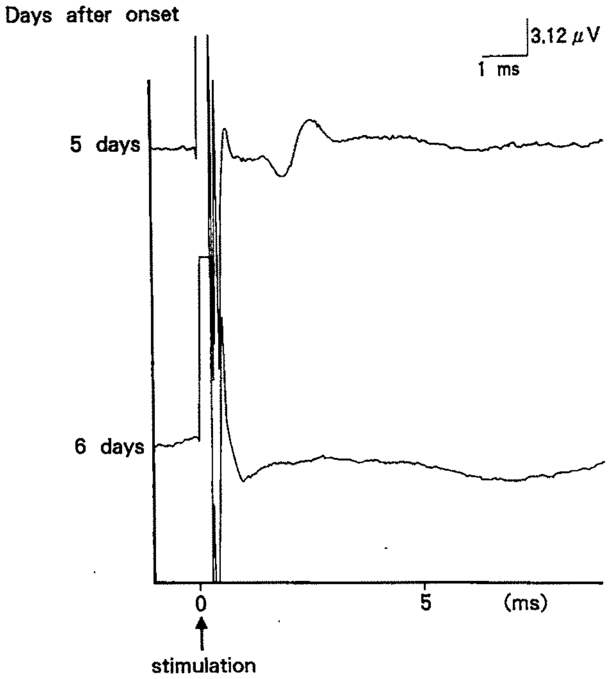

Fig 7. (Case 2) Waveform changes in patient with incomplete recovery. At 6 days after onset, AFNR changed to no response, whereas findings on electroneurography and nerve excitability test were still normal.

ter paralysis.

Case 2. A 64-year-old man was referred to our hospital for right facial paralysis of 3 days' duration. The findings of otoscopic examination, audiometry, vestibular function testing, and laboratory tests were all normal. There was no remarkable increase in the paired serum antibody titers of VZV or HSV-I. His facial movement was 12/40 on the Japanese grading system[15] and grade IV on the House-Brackmann system.[16] The right stapedial reflex was positive, and the lacrimation of the right eye was 307% of that of the left eye. The score on ENoG was 85.8%, and the NET findings were also normal. The AFNR revealed a biphasic wave, and the amplitude was 5.21 µV (Fig 7). Steroid therapy was started at our outpatient clinic, but the AFNR showed no response on the following day. At that time, the ENoG score was 32.6%, and the NET findings were still normal. The recovery of facial movement was poor, and the facial movement after 1 year was 28/40 on the Japanese grading system and grade III on the House-Brackmann system.

DISCUSSION

The AFNR is the only method of monitoring a nerve action potential, among all the electrodiagnostic tests of the facial nerve. The conventional tests, such as NET, ENoG, and the maximal stimulation test, utilize the response of the facial muscles as an indicator of facial nerve damage. As the facial nerve is stimulated out of the temporal bone in these tests, the evaluation of nerve function is limited to the extratemporal facial nerve. In Bell's palsy, the initial lesion is thought to be more proximal than the geniculate ganglion,[17] and the nerve degeneration extends to the peripheral part in severely damaged cases. Therefore, abnormal findings from these tests are obtained after most nerve fibers have already degenerated to the extratemporal segment of the facial nerve. According to previous studies, abnormal findings obtained from these tests appear more than 1 week after the onset of paralysis.[10-13] Therefore, if we desire to detect nerve degeneration during its early stages, it is necessary to use a test that can diagnose degeneration within 1 week after the onset of paralysis.

Since the AFNR represents intratemporal facial nerve function, it has the possibility to diagnose nerve degeneration during the early stages of paralysis. Therefore, we investigated whether this test could detect the initial signs of degeneration within 1 week after onset, by comparing findings obtained from patients with complete recovery with those from patients with incomplete recovery.

In the present study, no patient showed the triphasic wave that indicates normal conductivity in the peripheral nerve. This finding indicates that the AFNR accurately represents a nerve conduction block within the first week of paralysis. However, the abnormal responses do not indicate the existence of nerve degeneration, because there can be a reversible conduction block without nerve degeneration (neurapraxia).

Some findings obtained in the present study suggest that it is possible to detect degeneration of the intratemporal facial nerve by AFNR. On the first recordings, biphasic waves were recorded from 92 of the 109 patients. As previously mentioned, this waveform means that the site of nerve conduction block is proximal to the recording site. In our recording system, the recording electrode was placed at the posterosuperior part of the annulus tympanicus. Because the electrical potential recorded from the recording electrode reflects changes in the nerve action potential in the nearest part of the facial nerve, the obtained response indicates the electrical change from between the first and second genua.[9] Therefore, in patients who showed biphasic waves, the lesions might have been more proximal to the geniculate ganglion. Few studies have directly identified the lesion in Bell's palsy, which has been suggested to be proximal to the geniculate ganglion.[17-20] Fowler[18] found degeneration of the myelin sheath and axon cylinders of the facial nerve proximal to the geniculate ganglion and in the internal auditory canal in a patient with Bell's palsy 14 days after onset. McKeever et al[19] and Michaels[20] also reported pathological findings of the facial nerve in the internal auditory canal. Fisch and Esslen[17] observed a pronounced edematous, red swelling of the facial nerve proximal to the geniculate ganglion in 11 of 12 patients during total exposure of the facial nerve by the middle cranial fossa approach. If these studies represent the real pathological changes of Bell's palsy, monophasic waves and flat waves, especially seen in patients with nerve degeneration, might indicate that the lesion extends from the part of the internal auditory canal close to the geniculate ganglion or the more peripheral part of the facial nerve. That is, a monophasic or flat wave suggests the existence of extensive nerve damage and might be an indicator of nerve degeneration. Moreover, the chronological change of the waveform was also thought to indicate that the degenerative lesion spreads peripherally, because the pattern of the waveform change was from a biphasic wave to a monophasic or flat wave in most cases. As the waveform changes happened within 1 week after the onset of paralysis, the AFNR might be a test that indicates nerve degeneration during its early stages.

In some patients, it was impossible to tell whether nerve degeneration had occurred, by only the waveform of the AFNR. Of the patients who were tested only once, 8 patients had monophasic or flat waves in group A, 1 patient had a biphasic wave in group B, and 4 patients had biphasic waves in group C. In the patients with flat waves in group A, the AFNR might have been too small to detect by our monitoring apparatus. Since the AFNR is a near-field–type potential,[8] it is preferable to place the recording electrode as close as possible to the facial nerve. In our preliminary study of 12 normal subjects,[21] the average amplitude of the AFNR evoked from the intratympanic electrode was 49.4 μV, ie, approximately fourfold that recorded at the tympanic annulus (13.2 μV). However, intratympanic recording is unsuitable for repeated tests, because local anesthesia of the external auditory canal also produces inner ear anesthesia via the perforation of the eardrum made by insertion of the needle electrode. Moreover, in patients with a very small response, it was sometimes difficult to tell whether the waveforms were biphasic or monophasic. Therefore, the patients who we decided had monophasic waves might actually have had biphasic waves. In a case in which the AFNR appears monophasic or flat on a recording at the tympanic annulus, we should record the AFNR in the tympanic cavity only once. As another method to differentiate nerve degeneration, we may use the facial score. Among patients who had complete recovery in this study, no patient had a facial score lower than 10 points. Therefore, we strongly suspect nerve degeneration in patients with scores lower than 10 points and with monophasic or flat waves.

There were 4 patients, including 3 patients with only 1 test, who had biphasic waves within the first week in group C. In all patients, the last recording date was 5 days after the onset of paralysis; however, 2 of them who were tested on the 8th day showed no response. Since no patient had the AFNR tested on the 6th or 7th day after onset, it might have been possible to obtain waveform changes from biphasic to monophasic or flat by the 7th day in these 4 patients. Frequent daily tests should be performed within 1 week after the onset of paralysis, if possible, especially in patients with low facial scores.

In the patients with partial degeneration in group B, the AFNR and facial score were similar to those in patients with total degeneration. These patients were few (4.6%) in the present study, but they have the possibility of undergoing decompression surgery under the diagnosis of severe nerve degeneration. The AFNR has other parameters, such as the amplitude of the nerve potential and the latency,[7] and these parameters usually change under the condition of nerve degeneration. Therefore, further analysis of the AFNR with these parameters might be helpful in diagnosing nerve degeneration.

The most important thing in the clinical application of AFNR as a prognostic test for facial paralysis is whether facial nerve decompression surgery is effective in patients with nerve degeneration, according to the findings from the AFNR. Adour and Swanson[22] observed postoperative facial nerve recovery in 23 patients with Bell's palsy who were treated with transmastoid decompression surgery after the NET showed evidence of nerve degeneration. They concluded that early or late facial nerve decompression was not of benefit in patients with Bell's palsy, because the postoperative course was uneventful in all but 1 case. May et al[23] also evaluated the prognostic significance of ENoG in predicting the possible benefit of transmastoid facial nerve surgical decompression in 25 patients with Bell's palsy. Their studies indicated that even when ENoG was used to select the patients who might benefit most from transmastoid surgery, the outcome in patients treated surgically was no better than that that occurs without treatment. However, these findings were based on transmastoid surgery. A study by Gantz et al[24] demonstrated that middle cranial decompression, proposed by Fisch and Esslen[17] and Fisch,[25] improves the chances of return of normal or near-normal facial function, even in patients who show 90% degeneration or more on ENoG. They concluded that ENoG in combination with voluntary electromyography successfully differentiated patients who would most likely return to normal from those who had a greater chance of long-term sequelae from Bell's palsy, if they underwent middle cranial decompression within 2 weeks of onset of total paralysis.[24]

Electroneurography certainly is a very useful test for evaluating nerve degeneration; however, it takes more than 1 week after the onset of paralysis for evaluation of nerve degeneration, as previously mentioned. In general, the value indicating severe nerve damage on ENoG is less than 10% to 30% of the amplitude of the normal side,[26] and in our cases there was no patient who met this criterion within 1 week. However, as in case 2, most patients with nerve degeneration show rapid deterioration of ENoG findings within 1 week. Therefore, if a patient has a monophasic or flat waveform on AFNR and rapid deterioration of the ENoG value, the likelihood of complete recovery by only conservative treatment is remote. On the basis of these ideas, we conclude that ENoG and AFNR are not alternatives, but complementary prognostic tests, and that they should be performed simultaneously to diagnose nerve degeneration. Facial nerve decompression should undoubtedly be performed as soon as possible, even in the form of middle cranial surgery, because the longer the operation is delayed, the more the proportion of irreversibly damaged nerve fibers (neurotmesis) increases. In the study of Gantz et al,[24] 14 of 34 patients who underwent surgical decompression within 14 days after the onset of paralysis had a House-Brackmann[16] grade of I, and the recovery of facial function in patients operated on within 1 week was better than that in patients operated on between 1 and 2 weeks. If the operation could be performed within 1 week, the results might be improved.

In addition, some studies[27-29] indicated the usefulness of transmastoid decompression in combination with hypertonic osmotic agents. Kishimoto et al[27] clarified the presence of a direct communicating route between the subarachnoid space and the interstitial space of the facial nerve by histologic examination of a case of subarachnoid hemorrhage. In this case, the blood was distributed from the subarachnoid space in the internal auditory canal to around the geniculate ganglion. The blood penetrated into the interstitial space of the facial nerve. Moreover, when India ink and horseradish peroxidase were injected into the subarachnoid space in guinea pigs, these dye agents were detected in the interstitial space of the facial nerve from the labyrinthine segment to the tympanic segment, especially surrounding the geniculate ganglion. Takeda et al[28] simultaneously measured the interstitial fluid pressure of the facial nerve and the cerebrospinal fluid (CSF) pressure using a servo-nulling system in guinea pigs. The pressure in the facial nerve fluctuated coincidently with CSF pressure, and both pressures changed together after injection of saline solution into the CSF space. These findings also indicated a communication between the CSF space and the interstitial space of the facial nerve. On the basis of these experimental findings, the same group investigated the effect of glycerol, mannitol, and low-molecular dextran on the interstitial fluid pressure of the facial nerve.[29] The interstitial fluid pressure was measured at the distal segment of the facial canal in guinea pigs, and both peroral administration of glycerol and intravenous administration of mannitol lowered it, as well as the CSF pressure. However, low-molecular dextran did not cause significant changes in the interstitial fluid pressure of the facial nerve. They concluded that the main cause of the reduction in the interstitial fluid pressure of the facial nerve was pressure transmission from the CSF space. Thus, they proposed to study the effects of hypertonic osmotic agents on damaged facial nerves for clinical application of these agents in treating facial nerve palsy.

From the previously mentioned studies, it appears that if the hypertonic osmotic agents can decrease the interstitial pressure of the facial nerve central to the geniculate ganglion, transmastoid decompression might be effective in combination with these drugs. Namely, the hypertonic osmotic agents might play a role in decompression of the facial nerve from the meatal foramen to the geniculate ganglion, and the transmastoid decompression might help decompress the nerve peripheral to the geniculate ganglion. Middle cranial decompression might be effective in patients with severe nerve damage; however, there are few patients who accept this surgery, and there are few otologists capable of performing it correctly without undue risk. An operation without patient acceptance or an operation impossible for most otologists is meaningless, even if it is a very effective treatment. Therefore, the effect of transmastoid decompression surgery together with use of a hypertonic osmotic agent in patients with nerve degeneration should be discussed.

The AFNR is not a perfect method to diagnose and treat Bell's palsy; however, it is practically impossible to predict nerve degeneration before it occurs, even by using all of the electrodiagnostic tests. Because the AFNR can provide information that cannot be obtained by conventional tests, it should be applied as a method to investigate facial paralysis during its early stages.

CONCLUSIONS

In patients with nerve degeneration, it is very important to detect signs of degeneration as early as possible, to enhance the effect of decompression surgery. The AFNR can reveal nerve degeneration within 1 week after the onset of paralysis. The AFNR is, therefore, recommended as a monitoring method of nerve degeneration during the early stages of facial paralysis and for evaluation of the results of transmastoid decompression surgery.

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ANTIMICROBIAL DEFENSIN PEPTIDES OF THE HUMAN NASAL MUCOSA

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Defensins, a prominent group of antimicrobial peptides, are an important component of the innate immune response, particularly at mucosal surfaces that are vulnerable to colonization by potential pathogens. The present study was undertaken to investigate the expression of defensins in inferior turbinate mucosa of normal subjects and inferior turbinate mucosa and nasal polyps of patients with chronic sinusitis. Expression of β-defensin 1 and 2 and α-defensin 5 and 6 messenger RNAs (mRNAs) was investigated by reverse transcriptase–polymerase chain reaction, and their expression level was semiquantitatively evaluated by dot blot hybridization. Immunohistochemical analysis was used for detection of α-defensins 1, 2, and 3 in tissue sections. β-Defensin 1 mRNA was expressed in all tissue samples, at levels that did not differ significantly. β-Defensin 2 mRNA was detected in the turbinate mucosa and nasal polyps of patients with chronic sinusitis, but not in normal mucosa. Its expression level was significantly higher in nasal polyps than in turbinate mucosa. α-Defensin 5 and 6 mRNAs were not expressed in any tissues, and α-defensins 1, 2, and 3 were detected in all tissue samples obtained from patients with chronic sinusitis. These results suggest that β-defensin 1 may play a constitutive role in nasal defenses, whereas α-defensins 1, 2, and 3 and β-defensin 2 may be induced in response to local infection or inflammation.

KEY WORDS — defensin, inferior turbinate, nasal polyp.

INTRODUCTION

Antimicrobial peptides are a recently discovered component of the innate immune system that is proposed to participate in the early host defense response against microorganisms. Defensins, a major family of antimicrobial peptides found in mammals, have a wide range of antimicrobial activity encompassing gram-positive and gram-negative bacteria, fungi, and viruses. Depending on the spacing of the cysteine residues and the connectivity of the disulfide bridges, defensins are classified into 2 families, α- and β-defensins.[1-4] In humans, 6 α-defensins have been described: 4 (HD-1, HD-2, HD-3, and HD-4) from neutrophils and 2 (HD-5 and HD-6) from Paneth cells of the small intestine.[5-8] β-Defensins have a wider cellular distribution than α-defensins. β-defensin 1 is expressed in the urogenital tract, female reproductive tract, and lung.[9,10] β-Defensin 2 has been demonstrated in the skin and bronchial mucosa.[11]

The nasal mucosa, being constantly exposed to environmental pathogens, is particularly vulnerable to infection. A major function of the nasal mucosa is to present a barrier to the penetration of pathogens and other potentially injurious agents from the environment into the body. These mucosal defense mechanisms of the nose depend on nasal airway secretions and their constituent proteins, which derive from epithelial cells, submucosal glands, blood vessels, and secretory cells resident in the mucosa.[12-14] However, little attention has been paid to the expression of defensin family members in human nasal mucosa, despite their importance in the mucosal defense system.

The present study was undertaken to look for evidence of the known human defensin subclasses in human nasal mucosa.

MATERIALS AND METHODS

Tissue Preparations. Tissue from normal inferior turbinates was obtained from 10 subjects (5 men and 5 women, 24 to 40 years of age) undergoing surgery for augmentation rhinoplasty. All of these subjects were free of nasal symptoms at the time of investigation, and rhinoscopy did not reveal any anatomic abnormalities or signs of mucosal injury.

Nasal polyps and inferior turbinate tissue were taken from 10 patients (8 men and 2 women, 20 to 50 years of age) undergoing endoscopic sinus surgery for chronic polypoid sinusitis. These patients were selected on the basis of a history of chronic

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PRIMER SEQUENCES AND POLYMERASE CHAIN REACTION CONDITIONS FOR DEFENSIN SUBCLASSES

Primer		*Annealing Temperature*
α-Defensin 5		
Sense:	5′-TGATGAGGCTACAACCCAGAAGC-3′	56°C
Antisense:	5′-GAACTGAATGCACTGCTTTGG-3′	
α-Defensin 6		
Sense:	5′-GCACTGTCATGGGTATTAACCACA-3′	58°C
Antisense:	5′-TCATCCCTCAGAGGCAGCAGAAT-3′	
β-Defensin 1		
Sense:	5′-GACCACTATAACTGCGTCAGCAG-3′	55°C
Antisense:	5′-AAAAAGTTCATTTCACTTCTGCGTC-3′	
β-Defensin 2		
Sense:	5′-CCAGCCATCAGCCATGACGGT-3′	56°C
Antisense:	5′-GGAGCCCTTTCTGAATCCGCA-3′	
Glyceraldehyde-3-phosphate dehydrogenase		
Sense:	5′-ATCTTCCAGGAGCGAGATCC-3′	57°C
Antisense:	5′-ACCACTGACACGTTGGCAGT-3′	

polypoid sinusitis of more than 3 years' duration. None of these patients had a history of nasal allergy, asthma, or aspirin sensitivity.

Three normal skin samples from the postauricular region and 2 from bronchial mucosa, used as positive controls for β-defensin 1 and 2 reverse transcriptase–polymerase chain reaction (RT-PCR), were obtained at ear surgery and lung surgery, respectively. Neutrophil samples purified by Polymorphprep (Nycomed Pharma AS, Oslo, Norway) served as a positive control for α-defensin 1, 2, and 3 immunohistochemical analysis. These studies were approved by the Korea University Hospital Ethics Committee.

All tissue samples were collected for both RT-PCR and immunohistochemical analysis. Evidence of production of α-defensins 5 and 6 and β-defensins 1 and 2 was sought by RT-PCR, followed by dot blot hybridization. Evidence of α-defensin 1, 2, and 3 production was sought by immunohistochemical analysis.

All inferior turbinate and nasal polyp tissues were cut into 2 portions. One portion was dissected, frozen in liquid nitrogen, and stored at −70°C for subsequent RNA isolation. For immunohistochemical analysis, another portion was fixed in 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline solution (PBS, pH 7.2) overnight at 4°C, rinsed in a sucrose solution in PBS, and snap frozen in liquid nitrogen before cryosectioning to 12-µm thickness on a Leica CM 1900 cryostat.

RNA Isolation, RT-PCR, and Probe Construction. The frozen tissues (50 to 100 mg) were homogenized in 1 mL of TRIzol Reagent (Gibco BRL, Grand Island, New York), and total RNA was isolated as described previously.[15] Four micrograms each of total RNA extracted from inferior turbinate and nasal polyp tissues were reverse-transcribed in 20 µL of a reaction mixture containing 2.5 U of Moloney murine leukemia virus RT (Gibco BRL) and 50 pmol of random hexanucleotides at 42°C for 60 minutes. A PCR was carried out in a DNA thermal cycler (Takara Co, Shiga, Japan). One microliter of complementary DNA (cDNA) was amplified in 25 µL of PCR mixture containing 0.125 U of Taq DNA polymerase and 12.5 pmol of each primer. The RNA integrity and the success of the RT reaction were monitored by PCR amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts. Negative controls consisted of omission of the RT enzyme from the cDNA synthesis for each specimen. Published DNA sequences for human α-defensins 5 and 6 and β-defensins 1 and 2 were used for the primer construction. The PCR primers were designed with the oligonucleotide analysis program Primers! (Williamstone Enterprises, Waltham, Massachusetts). The primer sequences of each defensin subclass and GAPDH used in this study are listed in the Table. The amplified PCR products were resolved in 2% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light. The specificity of each PCR product was verified by predicted size and DNA sequencing.

For probe construction, the resulting cDNA fragments were inserted into the linearized vector, pCR 2.1 (Invitrogen Corp, Carlsbad, California), excised with *EcoRI*, and labeled with a mixture of deoxyribonucleosides containing digoxigenin–deoxyuridine-5′-triphosphate (Boehringer Mannheim, Mannheim, Germany) by a random priming technique.

Semiquantitative Dot Blot Hybridization for β-Defensin 1 and 2 mRNAs. Samples that demonstrated

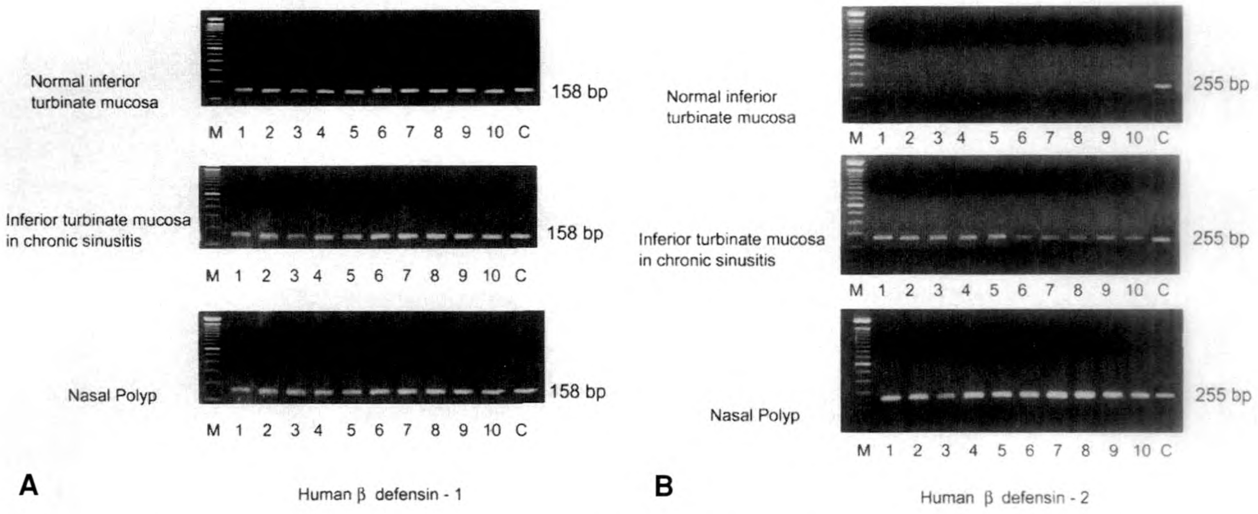

Fig 1. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of human β-defensin 1 and 2 messenger RNAs (mRNAs) in normal inferior turbinate mucosa obtained from rhinoplasty patients, and in inferior turbinate and nasal polyp tissue of patients with chronic polypoid sinusitis. **A**) Human β-defensin 1 complementary DNA (cDNA) segments with expected size of 158 bp were amplified by PCR for 35 cycles from all tissue samples. **B**) In contrast, human β-defensin 2 cDNA segments with expected size of 255 bp were amplified from inferior turbinate mucosa and nasal polyps of chronic sinusitis patients, but not from normal turbinate mucosa. Lane M — DNA marker; lane C — positive control: bronchial mucosa was used for β-defensin 1, and skin sample for β-defensin 2.

positive PCR reactions were further investigated by a dot blot hybridization assay. With this technique, we were able to semiquantify human β-defensin 1 and 2 mRNAs in the inferior turbinate tissues of the rhinoplasty patients and the inferior turbinate tissues and nasal polyps of the chronic polypoid sinusitis patients. The assay was performed in the following manner.

Total RNA was isolated and reverse-transcribed as described above. Each cDNA sample was processed by PCR by the individual primer pair and sampled at 20, 25, 30, 35, and 40 cycles. Time kinetic studies were performed to evaluate the optimal number of amplification cycles. On the basis of this analysis, 30 amplification cycles for GAPDH and 35 amplification cycles for β-defensins 1 and 2 were found to be optimally within the logarithmic amplification range.

Each aliquot of the PCR reaction was applied to a Hybond-N nylon membrane (Amersham, Buckinghamshire, England) by using a Bio-Rad dot blotting apparatus in accordance with the manufacturer's instructions. Sets of blots to be hybridized with individual probes were prehybridized at 68°C for 16 hours with 25 mL of hybridization solution (6× sodium citrate buffer, 1% sodium dodecyl sulfate, 5× Denhardt's solution, 10 μg denatured salmon sperm DNA) in a sealable bag. The probes were added to the prehybridization solution in a sealable bag, and hybridization was allowed to proceed for 24 hours at 68°C. Afterward, the hybridized blots were immunostained with anti-digoxigenin–alkaline phosphatase and visualized with the colorimetric substrate nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate.

The results were documented by photographing the wet filter and then scanning the photograph into a personal computer with an image scanner. The intensity of the individual dots was quantified with NIH image 1.57 software (Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland). GAPDH was taken as an indicator of the actual amount of cDNA used in each PCR. Therefore, the amount of target cDNA was quantified relative to the GAPDH level of each sample and expressed in relative units of each gene. Statistical significance was determined with the *t*-test. Differences between means were considered to be significant for p values less than .05.

Immunohistochemical Analysis. Cryostat sections were mounted on poly-L-lysine–coated slides and allowed to air-dry before immunohistochemical staining. To quench the endogenous peroxidase activity, we immersed the sections in methanol containing 0.03% hydrogen peroxide for 15 minutes. After being rinsed in PBS, the sections were incubated in 3% normal horse serum in PBS for 1 hour and thereafter rinsed again 3 times for 5 minutes each in PBS. They were then incubated with a mouse anti-human DEF 1-3 antibody (Bachem Inc, Torrance, California) at a dilution of 1:500 in PBS at 4°C overnight. For a negative control, an irrelevant mouse monoclonal IgG was diluted at the same protein concentration as

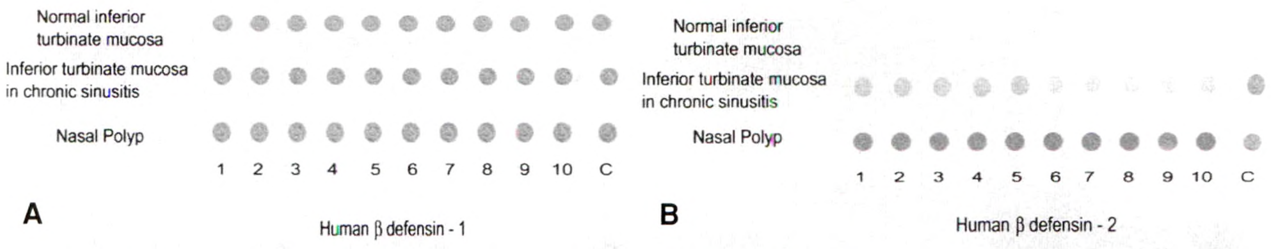
Normal inferior turbinate mucosa
Inferior turbinate mucosa in chronic sinusitis
Nasal Polyp
1 2 3 4 5 6 7 8 9 10 C
A
Human β defensin - 1
Normal inferior turbinate mucosa
Inferior turbinate mucosa in chronic sinusitis
Nasal Polyp
1 2 3 4 5 6 7 8 9 10 C
B
Human β defensin - 2

Fig 2. Dot-blot hybridization to detect and quantify **A)** human β-defensin 1 and **B)** human β-defensin 2 mRNA segments within turbinate mucosa obtained from rhinoplasty patients and turbinate and nasal polyp tissue of patients with chronic polypoid sinusitis. Lane C demonstrates positive control: bronchial mucosa was used for β-defensin 1, and skin sample for β-defensin 2.

that of the anti-DEF 1-3 antibody. The sections were then washed in PBS and incubated with biotin-labeled anti-IgG for 1 hour, and then with streptavidin-conjugated peroxidase for 1 hour (Vector Lab, Burlingame, California). Antibody binding was visualized with a hydrogen peroxide and diaminobenzidine reaction. The immunohistochemical sections were dehydrated in a graded ethanol series and mounted with Permount (Fisher Scientific, Fair Lawn, New Jersey).

To identify the cell types that produce α-defensins 1, 2, and 3 in nasal mucosa and polyps, we prepared several pairs of mirror image sections. One section in each set was immunostained for α-defensins 1, 2, and 3 as described above, and the other section was immunostained for neutrophil elastase (Dako, Carpinteria, California). Neutrophil elastase immunostaining was performed by the ABC method with rabbit polyclonal antibodies diluted 1:1,000 in PBS.

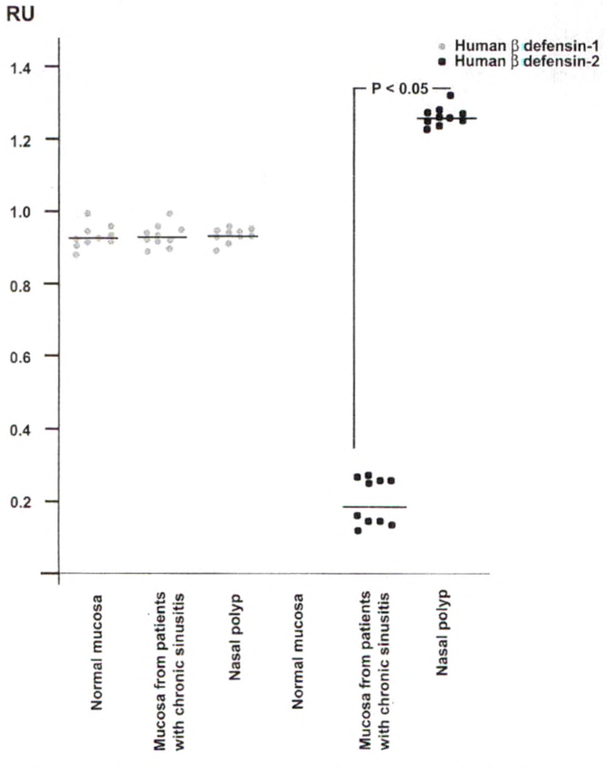

Fig 3. Semiquantitative analysis of human β-defensin 1 and 2 mRNAs in turbinate mucosa and nasal polyp. Total RNA was isolated and subjected to RT-PCR followed by dot blot hybridization. Exposed wet filters were scanned into personal computer by image scanner. Intensity of individual dots was quantified with NIH image 1.57 software. Amount of target cDNA was quantitated relative to glyceraldehyde-3-phosphate dehydrogenase level of each sample and expressed in relative units (RU) of each gene.

RESULTS

To investigate the pattern of defensin gene subclasses in human inferior turbinate mucosa and nasal polyp tissues, we used the sensitive technique RT-PCR. As a control for RNA integrity and the RT-PCR procedure, all RNA samples were analyzed for GAPDH expression, and all samples showed an amplification product of the expected size of 502 bp at apparently similar densities (not shown).

β-Defensin 1 RT-PCR of inferior turbinates and nasal polyps revealed a product at 158 bp in all samples with adequate cDNA yields (Fig 1A). Sequencing of this product revealed an almost exact match with the published sequence of the β-defensin 1 mRNA. β-Defensin 2 mRNA expression was detected in all nasal polyp tissues and turbinate samples obtained from patients with chronic sinusitis, but not in any normal turbinate tissues (Fig 1B). α-Defensin 5 and 6 mRNAs were not expressed in any samples tested.

In semiquantitative analysis with dot blot hybridization, no significant difference in β-defensin 1 mRNA levels was noted between turbinate tissues and nasal polyp tissues, whereas significantly higher levels of β-defensin 2 mRNA were expressed in nasal polyps than in turbinate tissues of patients with chronic polypoid sinusitis (Figs 2 and 3).

In inferior turbinate mucosa and nasal polyps obtained from subjects with chronic sinusitis, positive immunoreactivity for α-defensins 1, 2, and 3 was observed in inflammatory cells that had infiltrated into the epithelium and edematous stroma (Fig 4B,C). Mirror image sections using antibody against neu-

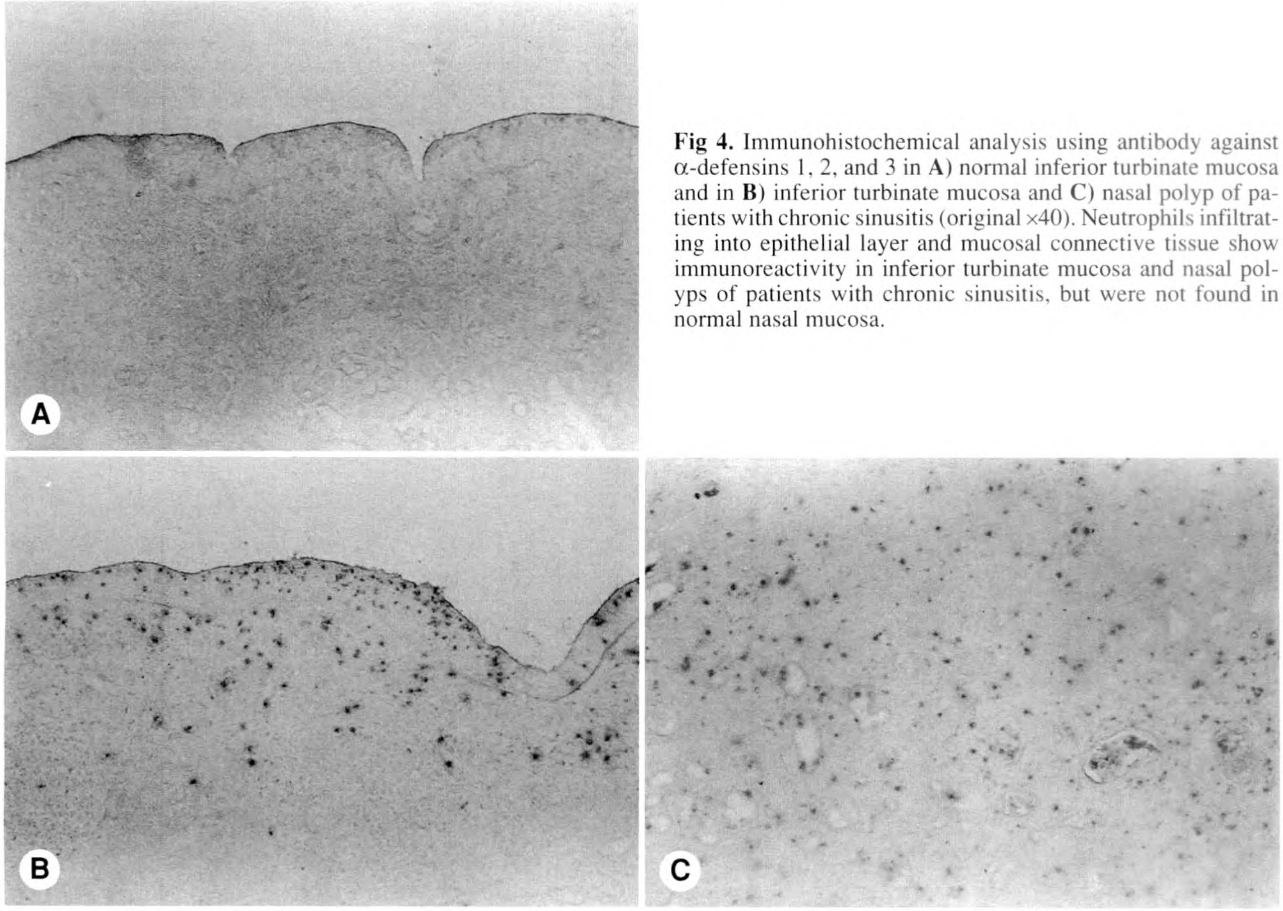

Fig 4. Immunohistochemical analysis using antibody against α-defensins 1, 2, and 3 in **A**) normal inferior turbinate mucosa and in **B**) inferior turbinate mucosa and **C**) nasal polyp of patients with chronic sinusitis (original ×40). Neutrophils infiltrating into epithelial layer and mucosal connective tissue show immunoreactivity in inferior turbinate mucosa and nasal polyps of patients with chronic sinusitis, but were not found in normal nasal mucosa.

trophil elastase revealed that the distribution of the α-defensin 1–, 2–, and 3–positive cells correlated best with neutrophil elastase–positive cells (Fig 5). Therefore, these results indicate that neutrophils express α-defensins 1, 2, and 3. However, normal turbinate mucosa obtained from 8 rhinoplasty patients failed to show positive immunoreactivity for α-defensins 1, 2, and 3 (Fig 5A). In the remaining 2 subjects, a few scattered immunoreactive cells were found in the inferior turbinate mucosa.

DISCUSSION

Nasal mucosa does not normally contain abundant phagocytic cells, as mentioned previously.[16,17] One would expect that epithelial cells and their secretions directly interfere with microbial colonization and invasion. Indeed, human nasal secretions contain a number of well-characterized antimicrobial products of surface epithelia and submucosal glands, including lysozyme, lactoferrin, secretory leukoprotease inhibitor, and secretory IgA. It has become clear that these secretory products provide the host with a primary defense against microbial infection.[12-14] Further, our data suggest that the human nasal mucosa innately produces a spectrum of antibiotic defensin peptides. It appears that β-defensins 1 and 2 are actually produced by the nasal mucosa and that α-defensins 1, 2, and 3 are largely released into the nasal secretions by resident or passing neutrophils. α-Defensins 5 and 6 were not detected in human nasal mucosa; thus, according to currently available information, defensins 5 and 6 are the sole product of small intestine Paneth cells. These results represent the first data to reveal defensin production by human nasal mucosa and implicate defensins in the innate protection of the human nasal mucosa.

All data available thus far on the defensin molecules present at mucosal surfaces indicate an important role in defense against microbial invasion. An understanding of the exact mechanism of production and regulation of defensins at the nasal mucosal surface will provide further insight into the occurrence of acute or chronic rhinosinusitis. One study evaluated the constituents of the nasal secretions obtained from patients with recurrent sinusitis. Baseline washings of patients with sinusitis had an abnormally high concentration of lysozyme and lactoferrin in relation to total protein — a finding suggesting that these antimicrobial factors were up-regulated in patients with chronic inflammation.[18] In the mucosa from patients with recurrent or chronic sinusitis, an increased immunoreactivity for lysozyme and lactoferrin was

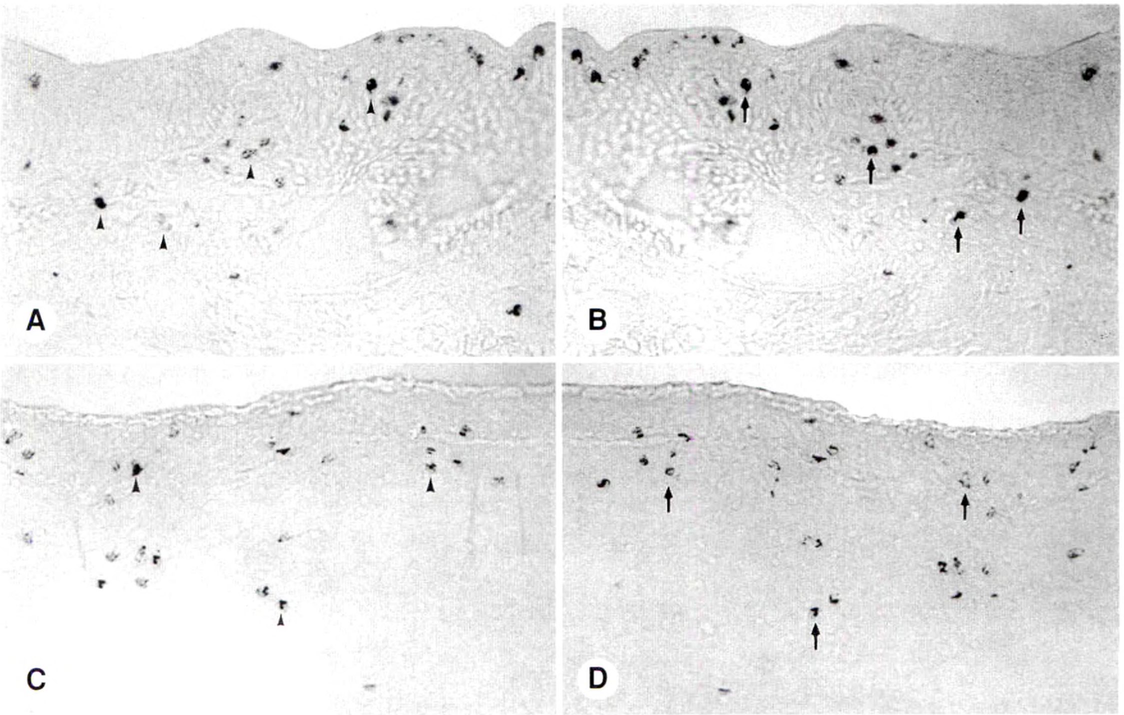

Fig 5. Distribution of α-defensin 1–, 2–, and 3–positive cells in **A)** turbinate mucosa and **C)** nasal polyps (original ×250). **B,D)** Results obtained with set of mirror-image sections: immunostaining with rabbit anti-neutrophil elastase antibody in **B)** turbinate mucosa and **D)** nasal polyps (original ×250). Majority of α-defensin 1–, 2–, and 3–producing cells (arrowheads) were cells with strong immunostaining for neutrophil elastase (arrows).

noted in epithelial cells and newly formed atypical glands.[19] Kurono et al[20] reported that the adherence of *Streptococcus pyogenes* to nasal mucosal cells was significantly greater in cells from patients with chronic sinusitis than in those from normal subjects, and that it was enhanced in the group with no antibody activity of secretory IgA against M protein. From these investigations, it can be considered that alteration in the activity or distribution of these antimicrobial factors has some association with the affinity of bacteria to epithelial cells, although it remains unclear whether the association is direct or indirect. Therefore, an important remaining question is whether the activity or expression of β-defensin 1 and 2 mRNA may be altered in states such as acute or chronic rhinosinusitis.

In the present study, β-defensin 1 mRNA was expressed in normal inferior turbinate, inflamed turbinate, and nasal polyp tissues. Semiquantitative dot blot hybridization revealed no significant difference in β-defensin 1 mRNA levels between normal and inflamed tissues. In contrast, β-defensin 2 mRNA was not expressed in normal inferior turbinate tissues, but was highly expressed in diseased nasal mucosa. These results are consistent with those of previous reports.[11,21-23] The relative expression of β-defensin 1 mRNA analyzed by RT-PCR was similar in non-inflamed and inflamed gingival tissues. Further, the expression of human β-defensin 1 mRNA in cultured gingival epithelial cells and tissues was constitutive and was not significantly modulated by cytokine- or bacterium-mediated stimulation.[22] In contrast, β-defensin 2 expression was induced markedly by interleukin-1β, and to a lesser extent by lipopolysaccharide.[21] Harder et al[11] showed that β-defensin 2 expression was induced in skin keratinocytes in the presence of tumor necrosis factor α, gram-negative and gram-positive bacteria, and *Candida albicans*. Taking these findings together, we speculate that β-defensin 1 plays a constitutive role in nasal defenses, whereas β-defensin 2 expression is induced in response to local infection or inflammation.

In the nasal mucosa of patients with chronic sinusitis, immunoreactivity of α-defensins 1, 2, and 3 was observed in neutrophils. Upregulation of α-defensins 1, 2, and 3 has been noted in purulent sputum from patients with cystic fibrosis or chronic obstructive pulmonary disease — conditions that are associated with markedly elevated neutrophil counts.[23] Another immunohistochemical study has demonstrated that human α-defensin 1, 2, and 3 mRNAs and proteins are not detected in normal human corneal stroma, except in a few inflammatory cells.[24] If these findings are taken together, the results of the present study indicate that human α-defensin 1, 2, and 3 expression may be enhanced by a number of neutrophils infiltrating sites of infection or inflammation and may inhibit further spread of microorganisms in human nasal mucosa. Additionally, α-defensins 1, 2, and 3 have been shown to be cytotoxic toward bronchial epithelial cells and stimulate interleukin-8 synthesis, mediating the recruitment of additional neutrophils into the airways.[25] Therefore, α-defensins 1, 2, and 3 may also contribute to the pathophysiological consequences of chronic rhinosinusitis in addition to their role in nasal mucosal defense

In conclusion, the results of the present study show that human β-defensin 1 mRNA is constitutively expressed in normal and inflamed nasal mucosae. Human β-defensin 2 mRNA and α-defensin 1, 2, and 3 proteins are expressed in inflamed mucosa, but not in normal nasal mucosa. These results suggest that β-defensin 1 may play a constitutive role in nasal defenses, whereas α-defensins 1, 2, and 3 and β-defensin 2 may be induced in response to local infection or inflammation.

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IN VIVO MEASUREMENTS OF AN IMPROVED TRACHEOSTOMA VALVE BASED ON INHALATION

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An improved, inhalation-based tracheostoma valve (TSV) was designed to facilitate finger-free tracheoesophageal speech. In contrast to existing models, the TSV closes by means of strong inhalation (instead of exhalation) to reach the "speaking position." Air is inhaled through a small 1-way valve that allows unlimited phonation time. The device can be deliberately changed to the "breathing position" by a fast expiration. Experimental setups were used to measure in vitro and in vivo the performance of the inhalation TSV. In patients, the inhalation TSV was compared with existing TSVs on the following parameters: air volume used to close the TSVs, speaking time of the TSVs, and total air volume during exhalation through the TSVs. The inhalation TSV functions well in physiological ranges, is optimally adjustable, and is an improvement over existing devices. It makes continuous speech possible and saves as much as 22% of the total exhalation volume for speaking, in contrast to existing TSVs.

KEY WORDS — total laryngectomy, tracheostoma valve, voice prosthesis, voice rehabilitation.

As part of the rehabilitation treatment after laryngectomy, some laryngectomees use a tracheostoma valve (TSV) that is placed on their tracheostoma (Fig 1). These TSVs make finger-free speech possible; the tracheostoma can deliberately be closed with this device, thus guiding air into the esophagus through a shunt valve between the trachea and the esophagus. Soft tissue at the entrance of the esophagus (pseudoglottis) starts to vibrate and functions as new "vocal folds."

Since the introduction of the Blom-Singer TSV,[1] several other TSVs have been designed.[2-7] All of these TSVs are based on the mechanism of exhalation, which has some disadvantages. Some air is already spent before phonation starts, as this mechanism needs a strong exhalation for closure of the TSV. Furthermore, continuous phonation is impossible, because inhalation reopens the TSV. To solve these problems, we designed a new TSV based on an earlier study.[8] In contrast to existing TSVs, this device was closed by inhalation, thus saving air for phonation. An integrated 1-way valve was present for inhalation during phonation, making continuous speech possible. Although the TSV functioned well, its round valve moving in a round housing was susceptible to slant. Furthermore, the breathing resistance was rather high compared with that of existing TSVs. The construction of the TSV was complicated, which caused difficulties in reproducibility and made the TSV expensive to produce.

Consequently, we tried to optimize this inhalation concept by designing an improved inhalation TSV. The most important requirement was that the valve construction should be simple and reliable and have a low breathing resistance. This report presents the design and testing of the improved inhalation TSV, but it concentrates on in vivo measurements. The improved inhalation TSV and 2 existing TSVs based on exhalation were tested in 3 patients to investigate the improved TSV in terms of maximum speaking

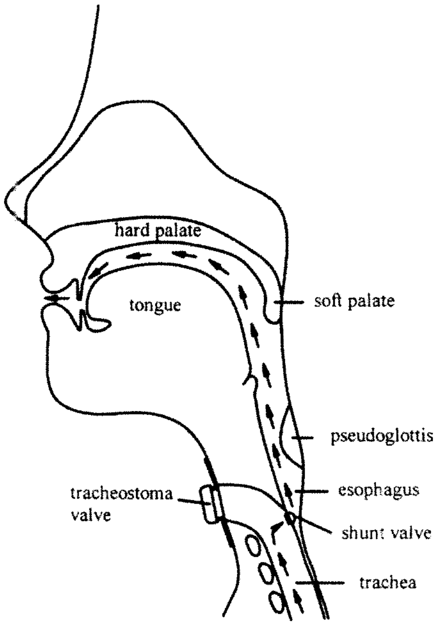

Fig 1. Rehabilitation after laryngectomy with shunt valve and tracheostoma valve (TSV).

From the Department of Biomedical Engineering, Faculty of Medical Sciences, University of Groningen, Groningen, the Netherlands. This study was part of the project "Voice Prosthesis" and was financially supported by the 4th Framework Program of the European Commission (Craft).

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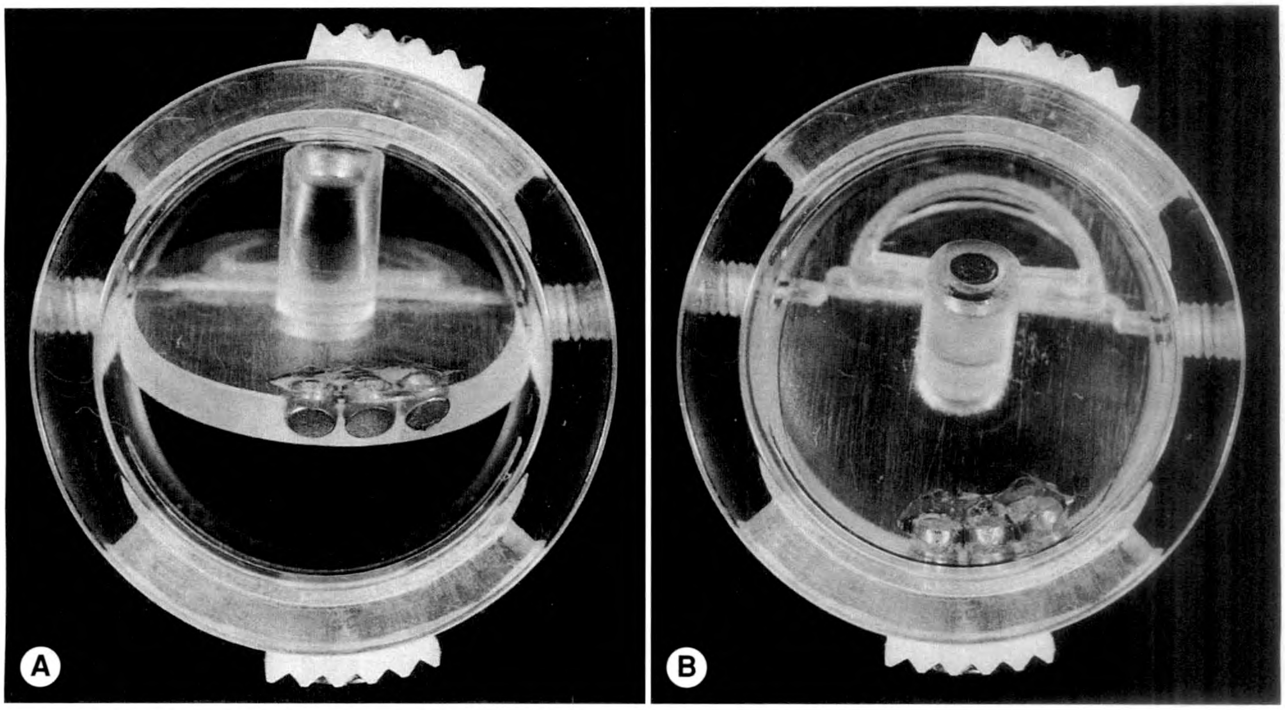

Fig 2. Inhalation TSV from top with **A**) open valve (breathing position) and **B**) closed valve (speaking position).

time and air volume left for speaking.

MATERIALS AND METHODS

Design. The inhalation TSV is depicted in Fig 2. It consists of a housing with an eccentric axis and a large valve that revolves on this axis (Fig 3). A small, half-moon–shaped silicone 1-way (inhalation) valve is integrated into the large valve. The TSV is made of polycarbonate, a very strong, but very light, size- and form-fixed material that resists weak acids very well and does not absorb liquid. The TSV can be attached to the stoma by housing it in a fixation ring that is attached to the skin with adhesives, double-sided foam or tape disks, and glue.[5]

The TSV is controlled by breath air and can be put in 2 positions: the breathing position and the speaking position. Normally, the large valve is in the breathing position and is kept open by the magnetic force between a metal plate on the housing and a magnet on the large valve. The large valve can be put in the speaking position by closing it by inhaling strongly. During exhalation, air is directed to the mouth through the shunt valve between the trachea and esophagus. In this speaking position, inhalation of air is possible through the small 1-way valve. This valve enables continuous speech, because the device stays in the speaking position. The TSV can be put back in the breathing position by a strong exhalation, which reopens the large valve.

Coughing is possible in both positions. In the breathing position, the TSV stays open, as a consequence of the inhalation mechanism. In the speaking position, the large valve is opened by the pressure produced by the lungs when coughing. Closure

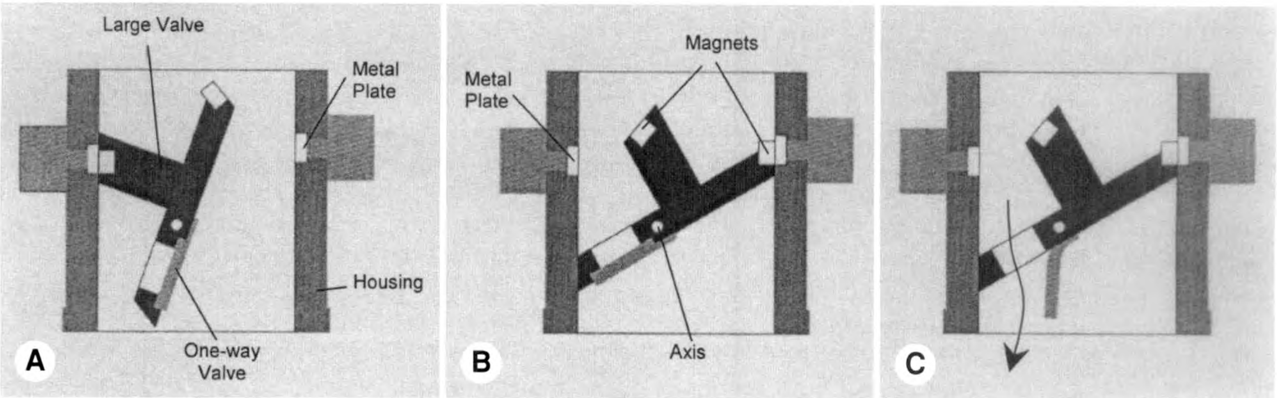

Fig 3. Cross-sections of inhalation TSV in **A**) breathing, **B**) speaking, and **C**) inhaling-during-speaking positions.

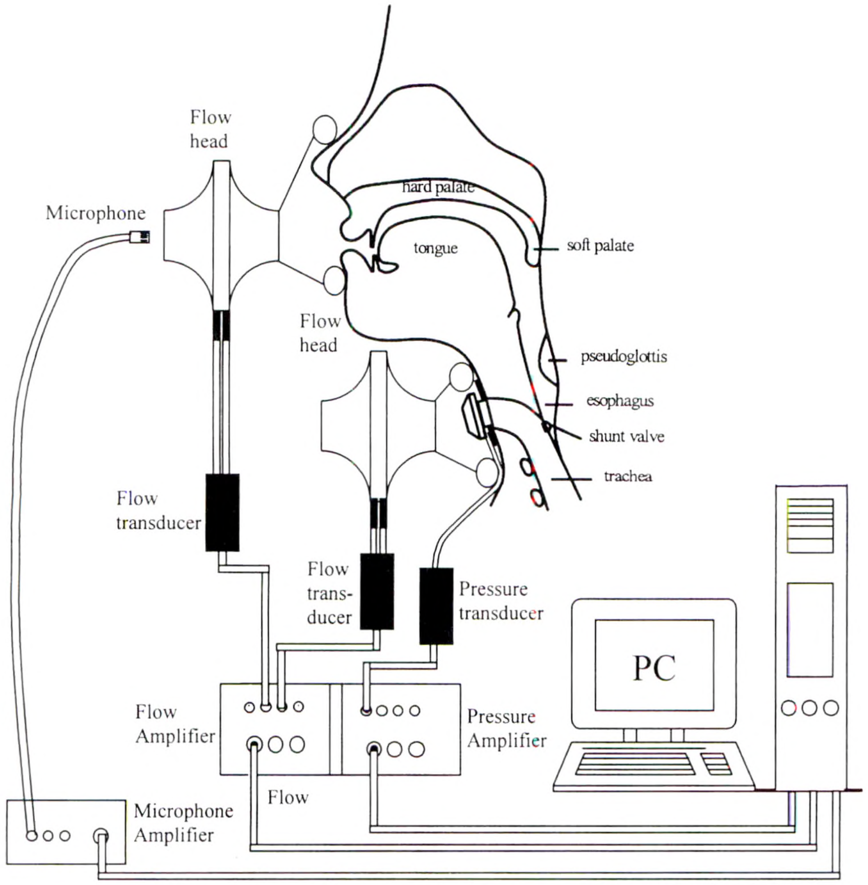

Fig 4. Setup to measure flow from stoma and mouth, pressure at stoma, and sound pressure level about 30 cm from mouth.

of the TSV can be adjusted by changing the magnetic force that keeps the TSV in the breathing position. The magnetic force is controlled by a slider that changes the contact area between the metal plate and the magnet. Another slider can adjust the opening of the TSV in the same way.

In Vitro Setup. To determine the flow values necessary to close the large valve and the pressure values necessary to reopen the valve, we developed an in vitro setup.[8] Flow and pressure were measured for the opening and closing of the large valve, respectively. The resistance of the TSV was determined by measuring the airflow resistance coefficient (ARC)[7] (also Verkerke et al, unpublished observations).

The ranges of closing flow and opening pressure were subdivided into 4 positions and 5 positions, respectively, with equal intervals between the positions. Three measurements per position were performed. Additional measurements were performed to calculate the ARC of the TSV during breathing. The ARC is calculated as follows: $p1 - p2 = ARC \cdot F^2$, where $p1 - p2$ is the pressure difference (over the TSV) and F^2 is the squared flow value. The measurements were made during breathing while the pressure difference over the TSV and the flow were registered simultaneously. To determine the ARC of the TSV, we plotted the pressure against the squared flow. A straight line was calculated with the linear regression approach, and the slope of the straight line represented the ARC. The value of the ARC for the breathing position differs from that for the speaking position, as inhaled air in the speaking position could only flow through the small 1-way valve, so that the ARC was increased. Accordingly, the ARC was calculated separately for the breathing and speaking positions. The ARC of the TSV was established for a flow range of −1.2 to 1.2 L/s and −0.6 to 0 L/s, respectively, for the breathing position and the speaking position. A negative flow indicated inhalation, and a positive flow indicated expiration.

In Vivo Setup. Three tests were performed to compare the improved inhalation TSV with commercially available TSVs. The following 3 TSVs were tested: 1) the Adeva Window TSV (Adeva Medical, Lübeck, Germany); 2) the Blom-Singer adjustable tracheostoma valve (ATV, Inhealth, Carpinteria, California); and 3) the inhalation TSV. The Adeva Window was tested in 3 positions, with position 1 the easiest position, position 2 the middle position, and position 3 the most difficult position in which to close the valve. The Blom-Singer ATV was tested in 2 positions, with position 1 the easier position and position 2 the more difficult position in which to close the valve. The inhalation TSV was also tested in 2 positions; position

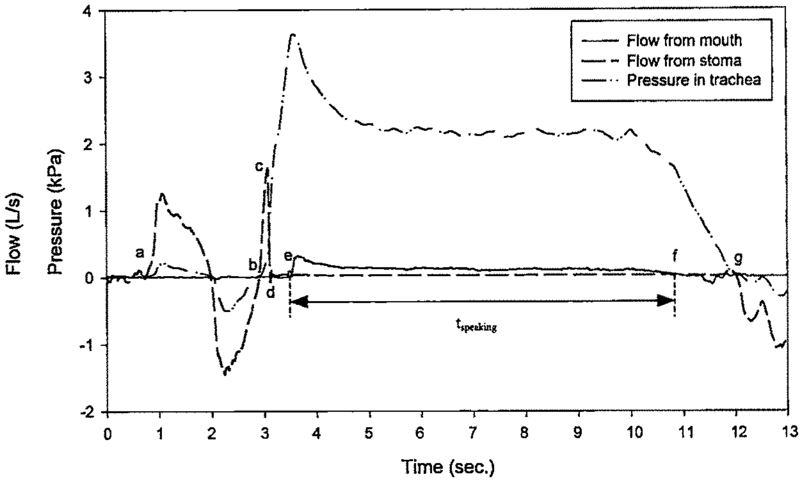

Fig 5. Measured flow from mouth (FM), flow from stoma (FS), and pressure in trachea (PT) during breathing and speaking plotted in one graph. Breathing starts at point a. At point b, quick exhalation starts, which closes TSV at point d. At point c, PT increases quickly, as TSV is closing. Speaking starts at point e, as FM becomes greater than zero. FM becomes zero again at point f, indicating that speaking has ended. At point g, TSV opens again and breathing starts.

1 is an easier position than position 2. All 3 patients possessed the ATV, with which they could speak well. The in vivo setup used is illustrated in Fig 4. Flow from the mouth, FM, was registered by a flow head connected to a mask held against the face of the patient. A thin catheter leading to a pressure transducer measured the pressure in the trachea, PT. The flow from the stoma, FS, was measured by a flow head connected to an adapter that was held over the tracheostoma.

The patients were asked to successively breathe with the TSV and then close the TSV and phonate as long as possible. An example of the resulting graph is shown in Fig 5. Three parameters were defined to analyze the performance of the 3 TSVs: 1) the speaking time after TSV closure, 2) the percentage of air volume spent to close the TSV, and 3) the total air volume during exhalation.

The speaking time ($t_{speaking} = t_e - t_f$; see Fig 5) after closure of the TSV is defined as the time that PT is greater than 1 kPa and FM is greater than zero. The percentage of volume that is spent to close the TSV is called the $V_{closing}/V_{total}$ ratio. The total air volume, V_{total}, is defined as $V_{total} = V_{closing} + V_{speaking} + V_{leakage}$. $V_{closing}$ is the volume from the stoma needed to close the TSV, calculated as

$$V_{closing} = \int_b^d Fs \bullet dt$$

This determines the air that is already spent before speaking starts. In contrast to the commercially available TSVs, $V_{closing}$ is negative for the inhalation TSV and is therefore not included in V_{total}. $V_{speaking}$ is the volume that leaves the mouth during speaking, calculated as

$$V_{speaking} = \int_e^f FM \bullet dt$$

$V_{leakage}$ is the volume leaking out of the stoma during speaking, calculated as

$$V_{leakage} = \int_d^g Fs \bullet dt$$

Student's *t*-test was used for statistical analysis ($p < .05$).

RESULTS

In Vitro. The airflow necessary to close the inhalation TSV is shown in Fig 6A. The airflow ranges from 3.8 to 1.2 L/s and decreases linearly from position 1 to position 4. Figure 6B shows the opening pressure of the TSV, which ranges from 7.1 to 1.2 kPa. These pressure values also decrease linearly from position 1 to position 5. The ARC values of the TSV in the breathing and speaking positions are displayed in Fig 7. The value of ARC during breathing is $ARC_{breathing} = 0.6 \bullet 10^2\ Pa \bullet s^2 \bullet L^{-2}$. The value of ARC of inhalation in the speaking position is $ARC_{speaking} = 22.7 \bullet 10^2\ Pa \bullet s^2 \bullet L^{-2}$.

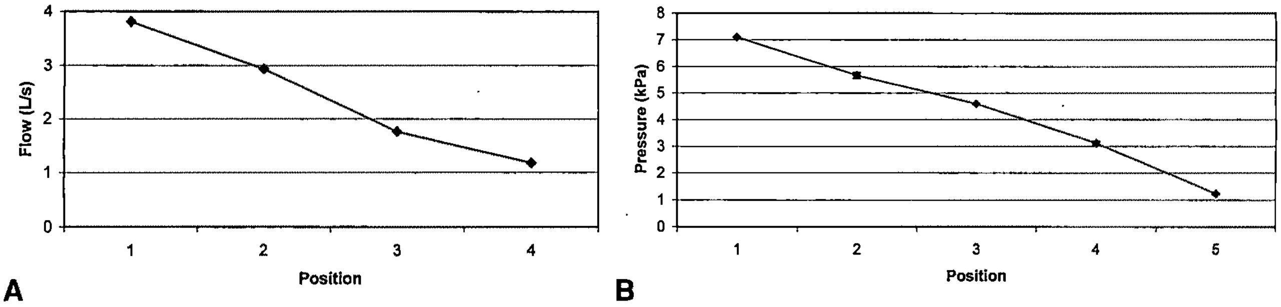

Fig 6. Values obtained with different valve positions (mean values based on 3 measurements per position) for **A**) flow required to close valve and **B**) pressure required to open valve.

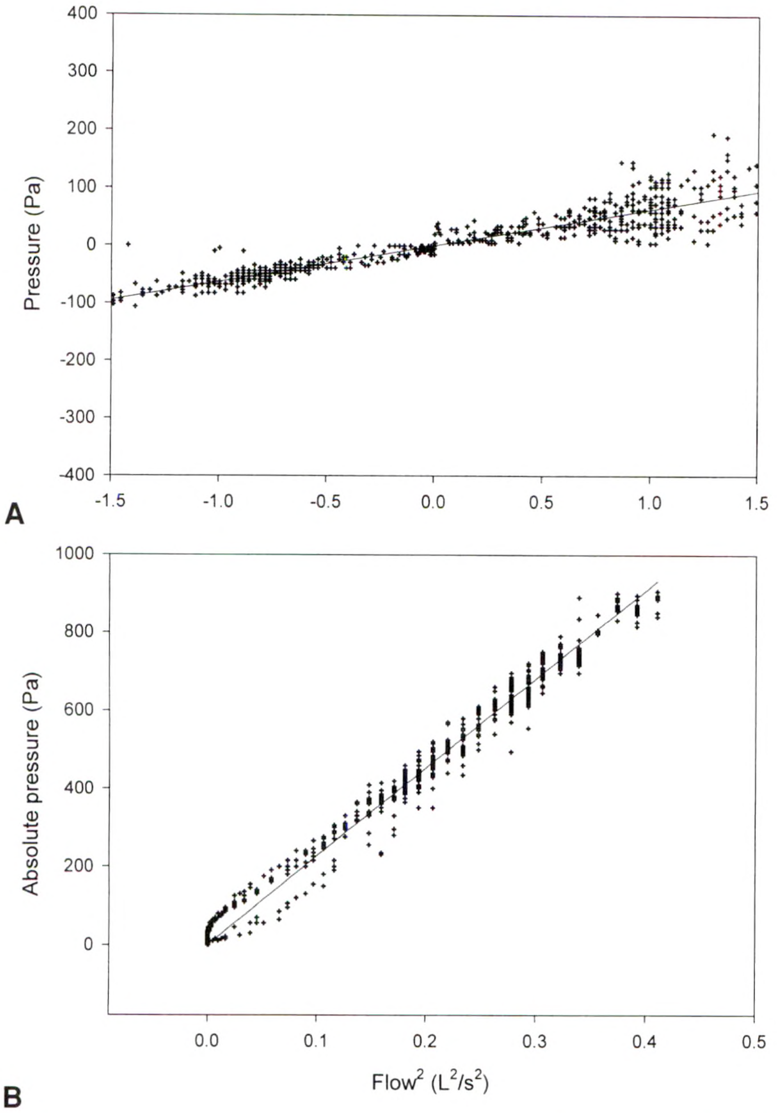

Fig 7. Pressure values plotted against squared flow values during **A**) inhalation and exhalation in breathing position and **B**) inhalation in speaking position. Straight line was obtained by linear regression. Slope of this line represents airflow resistance coefficient.

In Vivo. Two of the 3 patients were able to speak with the improved inhalation TSV very soon. Their first opinion of the inhalation valve was positive, but it appeared that it would take a few days for them to become completely used to the new mechanism. One patient was hardly able to speak with the inhalation TSV, as he could not control his breathing for speech very well. His results were excluded from the study. All patients could breathe easily with the inhalation TSV. The patients were able to close the inhalation TSV on command. The inhalation TSV could also be reopened on command when the correct adjustment was found. The position for reopening was adjusted to 4.4 kPa, which is greater than the tracheal pressure during speech. According to the patients, the biggest advantage is the ability to inhale while speaking. Another mentioned advantage is the ability to cough without TSV closure. A disadvantage is the size of the 1-way valve. Although the patients were able to inhale sufficient air for continuous speaking, 1 patient rated inhaling to be difficult.

The Table shows the values of the 3 parameters, $t_{speaking}$, V_{total}, and $V_{closing}/V_{total}$ ratio, that determine the performance of the TSV in vivo. Also, the parameters that determine V_{total} ($V_{closing}$, $V_{speaking}$, and $V_{leakage}$) are presented.

A statistically significant difference in $t_{speaking}$ was only found between the Adeva Window TSV in position 1 and the Blom-Singer ATV in position 2. The absolute value of $V_{closing}$ was 0.2 L for both the ATV and the inhalation TSV in both positions. The value of $V_{closing}$ of the Adeva Window increased from 0.2 L in position 1 to 0.4 L in position 3, with equal steps of 0.1 L, but the differences in $V_{closing}$ values were not statistically significant. The value of $V_{speaking}$ varied from 1.2 L for the Adeva Window to 1.6 L for the ATV. The differences in $V_{speaking}$ were only statistically significant for the comparison between the ATV (position 2) and the Adeva Window (positions 1, 2, and 3).

The value of $V_{leakage}$ was the highest in the inhala-

PARAMETERS FOR EVALUATION OF TRACHEOSTOMA VALVES

TSV and Position	$t_{speaking}$ *(s)*	$V_{closing}$ *(L)*	$V_{speaking}$ *(L)*	$V_{leakage}$ *(L)*	V_{total} *(L)*	$V_{closing}/V_{total}$	*n*
Adeva Window							
Position 1	6.1 ± 1.7	0.2 ± 0.1	1.3 ± 0.2	0.3 ± 0.4	1.8 ± 0.5	0.11	11
Position 2	6.9 ± 3.7	0.3 ± 0.1	1.2 ± 0.4	0.3 ± 0.3	1.8 ± 0.3	0.17	10
Position 3	6.6 ± 3.8	0.4 ± 0.2	1.2 ± 0.5	0.2 ± 0.3	1.8 ± 0.3	0.22	13
Blom-Singer ATV							
Position 1	7.6 ± 2.0	0.2 ± 0.1	1.4 ± 0.3	0.1 ± 0.1	1.6 ± 0.3	0.13	15
Position 2	9.5 ± 4.5	0.2 ± 0.1	1.6 ± 0.3	0.1 ± 0.2	1.9 ± 0.4	0.11	11
Inhalation TSV							
Position 1	7.0 ± 4.2	−0.2 ± 0.1	1.3 ± 0.3	0.5 ± 0.2	1.8 ± 0.5	0.00	6
Position 2	7.7 ± 3.5	−0.2 ± 0.1	1.5 ± 0.5	0.5 ± 0.4	2.0 ± 0.8	0.00	10

Data are mean ± SD.
TSV — tracheostoma valve.

tion TSV (0.5 L) and the lowest in the ATV (0.1 L). The differences in $V_{leakage}$ between the ATV and the inhalation TSV were statistically significant for all positions; the differences were not statistically significant between the Adeva Window and the inhalation TSV. The value of V_{total} varied between 1.6 L (ATV, position 1) and 2.0 L (inhalation TSV, position 2). The only difference in V_{total} that was statistically significant was the difference between the ATV in position 1 and the ATV in position 2.

The proportion of the total volume that is spent to close the TSV, the $V_{closing}/V_{total}$ ratio, varied from 11% (ATV in position 2 and Adeva Window in position 1) to 22% (Adeva Window in position 3). The $V_{closing}/V_{total}$ ratio is, for obvious reasons, zero for the inhalation TSV.

DISCUSSION

A tracheostoma is a severe consequence for patients who have undergone laryngectomy. The visible tracheostoma is embarrassing, especially when the patient wants to speak, because he or she has to point, in effect, to the handicap. A TSV can be a great improvement, as it makes finger-free speech possible.

In comparison with commercially available TSVs, 3 improvements can be distinguished in the inhalation TSV. The first is the possibility of continuous speech, which was said by the patients to be the biggest improvement over commercially available TSVs. The second improvement mentioned by the patients is that coughing is always possible. In commercially available devices (except for the Adeva Window), coughing causes closure of the valve, which sometimes leads to the very undesirable situation of the device's shooting out of the stoma. The third improvement is that no exhaled air is spent to close the valve. The in vivo measurements show that the value of the total exhalation volume, V_{total}, was nearly the same in the 3 different TSVs. Commercially available TSVs use as much as 22% of V_{total} to close the valve (see Table). Consequently, the main conclusion of the in vivo measurements is that the inhalation TSV saves as much as 22% of V_{total} for speaking.

This inhalation TSV is also an improvement in comparison with the former inhalation TSV.[8] The new TSV is more reliable because of its simple construction of a valve revolving on an axis, which also makes the TSV cheaper to produce. Its low weight and small size make wearing the device comfortable.

The flow needed to close the inhalation TSV and the opening pressure are optimally adjustable for the individual patient (Fig 6). It is important to adjust the opening pressure to just above the speech pressure so that opening the valve after speaking is not difficult. Although adjusting this pressure requires some time, the patients were able to find their individual opening pressures.

The ARC of the improved inhalation TSV in the breathing position is lower than that of the older TSVs,[4,7,8] making low-resistance breathing possible. The ARC in the speaking position, during inhalation through the small 1-way valve, is much higher. However, it is still 5 times lower than the resistance of the VoiceMaster prosthesis (Verkerke et al, unpublished observations), which is currently the shunt valve with the lowest resistance. Nevertheless, according to the patients' experience, this ARC value should be made lower. This can be done by resizing the 1-way valve.

It is remarkable that except between the Adeva Window in position 1 and the ATV in position 2, there is no statistically significant difference in speaking time ($t_{speaking}$) among the different TSVs for different positions. One might have expected a longer

$t_{speaking}$ in position 1 than in position 2 (and position 3). On the other hand, the fact that there is also no statistically significant difference in the volume necessary to close a TSV ($V_{closing}$) in different positions is in accordance with this phenomenon. The high SDs of $V_{closing}$ (as high as 50% of the mean) are the result of the fact that the value of $V_{closing}$ depends on the value of the airflow produced before closure. For example, when the airflow used to close the TSV is built up slowly, $V_{closing}$ can become very high.

The value of V_{total}, representing the total exhalation volume available after inhalation, should be the same for the different TSVs, but there was a statistically significant difference in V_{total} for the ATV in positions 1 and 2. Apparently, the patient inhales more deeply in position 2 before speaking, possibly in expectation of the greater effort needed to close the ATV in this position. The SD of V_{total} was rather high for the inhalation TSV, perhaps because patients were not used to the new mechanism and performed maneuvers that caused differences in measurements. According to the $V_{speaking}$ values, the ATV in position 2 has more volume for speech than the Adeva Window in positions 1, 2, and 3. This could be because the air that the Adeva Window loses during speaking, $V_{leakage}$, is greater than that of the ATV. The value of $V_{leakage}$ was the highest for the inhalation TSV. Consequently, it is important for further development of the inhalation TSV to minimize this leakage. The very high SDs of $V_{leakage}$ were caused by large differences in $V_{leakage}$ among the patients.

A special characteristic of the ATV is that the valve opens before all exhaled air is spent. The reason is that the ATV needs a certain pressure to stay closed, in contrast to the Adeva Window, which only opens during inhalation.

The effect of experience with a particular TSV should not be underestimated. The tested patients were accustomed to the ATV, and were trying the Adeva Window and the inhalation TSV for the first time. So, longer practice time might improve the results, especially for the inhalation TSV, which operates on a different principle.

Fixation of TSVs is still a major problem for many patients. Gluing a flange to the skin around the tracheostoma or using a Barton Mayo button that fits in the tracheostoma are the most often used methods for fixation of TSVs. However, these methods often fail because of air leakage. Research has been started on developing a new fixation method using an implantable permucosal device, the so-called tissue connector, to fix a tracheostoma valve to the trachea.[9]

The next step should be testing the inhalation TSV in larger series of patients for a longer period of time. An important addition to this inhalation TSV will be a heat and moisture exchanger, which moisturizes and heats the inhaled air, thus reducing respiratory symptoms.[10,11] Adeva Medical, which patented this improved inhalation TSV, will perform further development in preparation for putting it on the market.

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DETECTION OF CYTOKERATINS IN NORMAL AND MALIGNANT LARYNGEAL EPITHELIA BY MEANS OF REVERSE TRANSCRIPTASE–POLYMERASE CHAIN REACTION

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Cytokeratins (CKs) are a subgroup of intermediate filament proteins that take part in forming the cytoskeleton. The epithelial cells in different organs express distinct CKs, and this expression may be modified during malignant transformation. Here we describe the use of reverse transcriptase–polymerase chain reaction followed by Southern blotting to determine the profile of expression of CKs in both normal and malignant laryngeal samples. Thirty-six samples were subjected to histologic examination and classified as 17 squamous cell carcinomas, 3 dysplastic lesions, and 16 normal samples. CK8 and CK19 were expressed in almost all samples, both cancerous and normal, and were therefore used to verify the integrity of RNA. Expression of CK2, CK9, and CK20 was not detected in any of the samples, normal or cancerous. CK15 and CK18 showed low sensitivity for detection of cancer (36.4% and 45.5%, respectively). CK10 showed relatively high sensitivity (91%), but only moderate specificity (69.2%). Only CK17 showed both high sensitivity and specificity (91% and 92.3%, respectively; positive predictive value, 91%). We propose that CK17 may be considered a promising candidate to use as a molecular marker for malignant transformation in laryngeal squamous cell carcinoma.

KEY WORDS — cytokeratin, laryngeal carcinoma, reverse transcriptase–polymerase chain reaction.

INTRODUCTION

Head and neck malignancies account for 6.6% of all cancer cases in the United States — approximately 30,000 patients per year — and the most common of them (21%) is laryngeal cancer.[1] Squamous cell carcinoma (SCC) comprises about 95% of all laryngeal cancers. The treatment options include surgical resection, radiotherapy, chemotherapy, and combination therapy, and preservation of laryngeal function is now a main consideration in the choice of treatment.[2] Follow-up of these patients includes scheduled clinical examination and, if needed, endoscopy combined with biopsy to detect recurrence of the tumor as early as possible. Thus, current methods of follow-up place a high burden on the patient, as well as on the health care system. The use of biochemical markers for the detection and follow-up of laryngeal carcinoma could help to determine the prognosis of the patient, to control the progression of the lesion, and to avoid further discomfort for the patient.

The cytokeratins (CKs) are a family of small proteins, 20 of which have been characterized so far, that together constitute a subgroup of the intermediate filament proteins. Cytokeratins are distributed in all epithelial cells, take part in forming the cytoskeleton, and are responsible for the mechanical strength and structural stability of the cell.[3-5] Different types of epithelium express distinct sets of CKs, and neoplastic transformation has been shown to change this pattern of expression.[6,7] Previous studies have demonstrated the expression of several CKs in the larynx (CK4, CK5, CK7, CK8, CK13, CK14, CK15, CK17, CK18, and CK19).[4,8,9] In premalignant lesions, an increase in the levels of CK10, CK14, CK16, and CK17 and a decrease in the levels of CK4 and CK13 were found.[4] In this study, we describe the use of the reverse transcriptase–polymerase chain reaction (RT-PCR) to determine the profile of expression of CK genes in both normal and malignant laryngeal samples.

MATERIALS AND METHODS

Tissue Samples. We took 36 tissue samples (biopsy specimens) from 24 patients with suspected primary or recurrent laryngeal SCC. The specimens were obtained during suspension microlaryngoscopy or as part of a laryngeal operation by an external ap-

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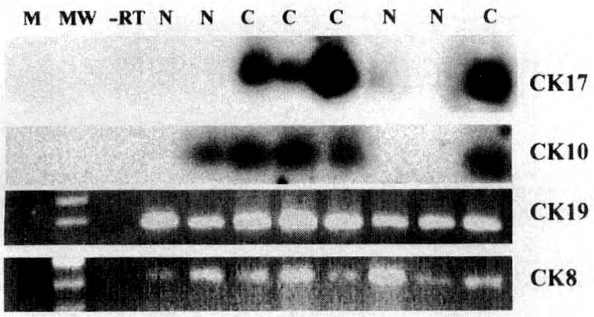
TABLE 1. SEQUENCES OF PRIMERS AND PROBES FOR EACH CYTOKERATIN

Cytokeratin	*Accession No.*	*Polymerase Chain Reaction Product (base pair)*		*Sequences*
CK2	M99063	601	Sense	5'- CGCACTGCCGCAGAGAATGAGTTTG
			Antisense	5'- CATCCAGGGCCAGCTTGACATTCATC
			Probe	5'- GCTCCTGGTAGTCACGCAGGAGCCGAGCCAGG
CK8		244	Sense	5'- AACAACCTTAGGCGGCAGCT
			Antisense	5'- GCCTGAGGAAGTTGATCTCG
CK9	Z29074	385	Sense	5'- TCACCAAGACCCTCAATGACATGCGTC
			Antisense	5'- AGCCGCATCTTAATGCTGAGCAGAAGG
			Probe	5'- CCTGA TTCTGGCACTCGATCTCTTGCCGGACG
CK10	X14487	131	Sense	5'- GCTGACCTGGAGATGCAAATTGAGAGCC
			Antisense	5'- GGGCAGCATTCATTTCCACATTCACATCAC
			Probe	5'- GGACACATTTCGAAGGTCTTTCATTTC
CK15	X07696	391	Sense	5'- TGAAGAGCTCCGGGACAAGATCATGG
			Antisense	5'- TTGCTGAAGAACCAGGCCTCGACATC
			Semi-antisense	5'-GGCGGTTCTTCTCCGCCATGGCCTCGTACTGC
			Probe	5'- CCCGGTGCTGCGTCCATCTCCACATTGACCTGG
CK17	Z19574	159	Sense	5'- TGAAGAAGAACCACGAGGAGGTGAGGTG
			Antisense	5'- ATCCTTGCGGTTCTTCTCTGCCATCTTC
			Probe	5'- GGTCACGCATCTCGTTGAGGATGCG GCTCAGG
CK18	X12883	287	Sense	5'- TACTGTGGACAATGCCCGCATCGTTC
			Antisense	5'- ATCTTGGCGAGGTCCTGAGATTTGGG
			Semi-antisense	5'- GCATCTACCTCCACGGTCAACCCAGAGCTGGC
			Probe	5'- GCCTCGATCTCTGTCTCCAGCTGCAGTCGTG
CK19		214	Sense	5'- GCGGGACAAGATTCTTGGTG
			Antisense	5'- CTTCAGGCCTTCGATCTGCAT
CK20		370	Sense	5'-CAGACACACGGTGAACTGG
			Antisense	5'- GATCAGCTTCCACTGTTAGACG
			Nested sense	5'-CTGTTTGTTGGCAATGAGAAAATGG
			Nested antisense	5'- GATCTCTCTCAGTCTCATA

proach. The specimens were taken from the tumor or from apparently normal mucosa remote from the tumor site. Each specimen was sliced in two: one part was taken for pathological examination, and the other was immediately frozen in liquid nitrogen to avoid RNA degradation. The RT-PCR was performed without knowledge of the histologic characterization of each sample.

RNA Extraction, RT-PCR, and Southern Blot Analysis. Total RNA was extracted from the biopsy specimens with TriReagent (Molecular Research Center, Inc, Cincinnati, Ohio) according to the manufacturer's instructions.[10] Five micrograms of the RNA were reverse-transcribed at 37°C for 1 hour with 200 µmol/L deoxynucleotides (Sigma, St Louis, Missouri), 5 µmol/L random hexamers, 20 U of RNA-guard, and 20 U/µg Moloney murine leukemia virus–RT (all from Amersham Pharmacia Biotech, Piscataway, New Jersey). Complementary DNA (cDNA) from each biopsy specimen was amplified separately for each CK by use of specific primers (Table 1). To increase the sensitivity of the method, we performed a semi-nested or nested polymerase chain reaction (PCR) amplification for CK15, CK18, and CK20. The PCR reaction included 200 µmol/L deoxynucleotides (Sigma), 0.5 µmol/L specific primers, 0.5 U of Taq Polymerase (Boehringer Mannheim, Mannheim, Germany), 500 ng of cDNA, and 0.5 µg of Flash Taq Polymerase monoclonal antibody (Chimerx, Milwaukee, Wisconsin). The PCR reaction was performed for 40 cycles; each cycle included 30 seconds of denaturation at 94°C, 30-second annealing at 55°C, and 30-second extension at 72°C. The PCR products were separated on 3% agarose wide range 3:1 (Sigma) and then transferred to a nylon membrane (Sartolon, Sartorius AG, Göttingen, Germany) by capillary blotting. Probes for CK2, CK9, CK10, CK15, CK17, and CK18 were 5'-end labeled with (γ-^{32}P) adenosine triphosphate by means of polynucleotide kinase (Boehringer Mannheim) to a specific activity of about 5×10^8 counts per minute per microgram. Membranes were hybridized to the specific probes at 42°C for 1 hour with Rapid-Hyb buffer (Amersham Life Sciences, Amersham International plc, Buckinghamshire, England). The membranes



Expression of cytokeratins in normal and cancerous samples. Amplification of CK8, CK19, CK10, and CK17 was performed as described in Methods, and separated on agarose gels. Southern blots were performed for CK10 and CK17. M — mix; MW — molecular weight marker ϕX174 HaeII digest; –RT — amplification of RNA without reverse transcription; N — normal samples; C — cancerous samples.

were washed in 1× SSC (sodium chloride and sodium citrate), 0.1% sodium dodecyl sulfate (SDS) for 20 minutes at room temperature, and 4 times in 0.1× SSC, 0.1% SDS for 15 minutes at 42°C. The membranes were autoradiographed with Kodak BMX film in the presence of intensifying screens and exposed at –70°C for 2 to 5 days.

Primers and Probes. Primers and probes were designed from the published sequences of the CKs (Table 1). Sequences for primers of CK8 and CK20 were adopted from the method of Rotem et al.[11] Sequences for primers of CK19 were adopted from the method of Burchill et al.[12]

Statistical Analyses. Sensitivity, specificity, and positive predictive values were calculated to assess the effectiveness of the markers as screening tools. Fisher's exact tests were calculated for comparing the normal samples versus the cancer samples in the unpaired analysis. For paired data, the McNemar test for two related dichotomous variables was used. For all analyses, the level of significance was $p \leq .05$.

RESULTS AND DISCUSSION

We have studied the expression of different CKs in both normal and malignant larynges in order to find a useful biochemical marker for the detection of laryngeal cancer. Of the 9 different CKs we have examined, only CK17 emerged as a possible candidate. The larynx contains several types of epithelium and is mostly covered by columnar ciliated epithelium. The true vocal cords and the laryngeal surface of the epiglottis are covered by stratified squamous epithelium, whereas pseudostratified epithelium is found in the ventricle.[13] The expression of different sets of CKs in epithelial cells has been shown to be specific for the type of epithelium; however, exogenous stimulators or malignant transformation may change this expression. For example, it has been shown that in smokers, the normal ciliated epithelium is often replaced by squamous epithelium.[13]

CK8 and CK19 were expressed in almost all samples, both cancerous and normal (see Figure and Table 2). Almost all samples categorized as SCC expressed CK8 (17 of 17) or CK19 (14 of 17). All samples categorized as dysplastic lesions expressed both CK8 and CK19 (3 of 3), and almost all of the normal samples expressed CK8 (13 of 16) and CK19 (16 of 16). Therefore, these CKs were used as controls to verify the integrity of the extracted RNA. CK8 and CK19 are usually referred to as markers of simple epithelium, but they are also expressed in stratified and pseudostratified epithelia,[4,8,14] and have been also detected in SCC lesions.[15] Accordingly, elevated levels of Cyfra 21-1, a product of the proteolytic cleavage of CK19, have been found in the serum of patients with head and neck SCC.[16] We have found that the expression of CK8 and CK19 genes in the larynx is constitutive, and thus, these molecules were excluded for use as markers of malignant transformation. Although their level of expression may be modified by malignant transformation,[16] our method of detection is not quantitative and did not examine this aspect.

In contrast to CK8 and CK19, we did not detect the expression of CK2, CK9, or CK20 messenger RNA (mRNA) in any of the samples, normal or cancerous (data not shown). CK2 is expressed mainly in stratified cornified epithelium, such as the gingiva and hard palate in the aerodigestive tract.[4] Expression of CK9 has been found only in the suprabasal layer of the palmar and plantar epidermis.[17] CK20 is expressed in normal gastrointestinal epithelium, in transitional urothelium, and in Merkel cells.[18] Expression of CK20 has been shown to serve as a marker for the detection of transitional cell carcinoma of the bladder[11,19] and of colon carcinoma.[12] Thus, these three CKs are not expressed in laryngeal epithelial cells, and their expression is not induced by malignant transformation.

CK15 is expressed mainly in the basal layer of stratified epithelium, and CK18 is found in both simple and pseudostratified epithelia.[4,8] Therefore, we examined their gene expression in the larynx, which includes these types of epithelium. CK15 was expressed in a minority of the premalignant and cancerous samples, exhibiting low sensitivity (36.4%). We have also found low sensitivity (45.5%) of the expression of CK18 in the larynx. The expression of CK18 in laryngeal cancer has scarcely been studied. In one study,[20] expression of CK18 was found in al-

TABLE 2. EXPRESSION OF CYTOKERATINS IN LARYNGEAL SAMPLES

Patient No.	*Age*	*Sex*	*Histology*	*Stage*	*Grade*	*CK10*		*CK17*		*CK19*		*CK8*		*CK15*		*CK18*	
						N	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*
1	61	M	SCC	T3N0M0	WD/MD	+	+	+	+	+	+	+	+	+	+	+	+
2	26	M	Normal	T3N0M0		–	NA	+	NA	+	NA	–	NA	–	NA	–	NA
3	82	M	Normal	T3N0M0		–	NA	–	NA	+	NA	+	NA	–	NA	+	NA
4	71	M	Dysplasia	T3N0M0		NA	+	NA	+	NA	+	NA	+	NA	–	NA	–
5	81	M	Dysplasia	T3N0M0		NA	+	NA	+	NA	+	NA	+	NA	–	NA	–
6	79	M	SCC	T3N0M0	WD/PD*	–	–	–	+	+	+	+	+	–	–	+	–
7	64	M	SCC	T1N0M0	MiCA	NA	+	NA	+	NA	+	NA	+	NA	–	NA	–
8	73	M	SCC	T3N2bM0	MD	–	–	–	–	+	–	+	+	–	–	–	–
9	53	M	SCC	T2N0M0	MD	NA	–	NA	–	NA	+	NA	+	NA	–	NA	–
10	67	M	SCC	T4N1M0	WD/PD	–	–	–	+	+	+	+	+	–	–	–	–
11	72	M	SCC	T2N0M0	MD	NA	+	NA	+	NA	+	NA	+	NA	+	NA	+
12	55	M	Dysplasia			NA	+	NA	+	NA	+	NA	+	NA	+	NA	+
13	53	F	Normal			+	NA	–	NA	+	NA	+	NA	+	NA	+	NA
14	64	M	SCC	T4N2cM0	WD	NA	+	NA	+	NA	+	NA	+	NA	+	NA	+
15	67	M	SCC	T3N0M0	WD/PD	+	+	–	+	+	+	+	+	+	–	–	–
16	77	M	SCC	T1bN0M0	WD/MD*	NA	+	NA	+	NA	–	NA	+	NA	–	NA	–
17	57	M	SCC	T1bN0M0	WD	+	+	+	+	+	+	+	+	–	–	+	+
18	53	M	SCC	T4N0M0	WD	+	+	–	+	+	–	–	+	–	–	–	–
19	70	M	SCC	T3N0M0	WD/MD	–	+	–	+	+	+	+	+	–	+	–	+
20	77	M	SCC	T1bN0M0	MD/PD	–	+	–	+	+	+	+	+	–	–	+	+
21	77	M	SCC	T2N0M0	MD*	–	–	–	–	+	+	+	+	–	–	–	–
22	79	M	Normal			+	NA	–	NA	+	NA	+	NA	–	NA	+	NA
23	84	M	SCC	T2N0M0	WD/MD*	–	+	–	+	+	+	–	+	–	–	–	–
24	94	M	SCC	T2N0M0	MD*	–	–	–	–	+	+	+	+	+	+	+	+

CK2, CK9, and CK20 were tested and were found negative for all samples, normal and cancerous.

N — samples taken from normal mucosa; C — samples taken from laryngeal lesions; SCC — squamous cell carcinoma; WD — well differentiated; MD — moderately differentiated; NA — not available; PD — poorly differentiated; MiCA — microinvasive carcinoma.

*Biopsy specimen was obtained after radiotherapy.

most all samples of SCC of the larynx and hypopharynx (11 of 12 samples), whereas in another study,[21] 17 of 37 laryngeal SCC samples showed extensive staining of CK18 with monoclonal antibodies. Thus, the expression of CK18 and CK15 in laryngeal epithelial cells warrants further investigation.

Expression of CK10 and CK17 in the larynx has hardly been studied. Expression of CK17 has been found in only 1 sample of SCC of the epiglottis, while no expression of CK10 was detected.[8] Another study showed a correlation between cornification and expression of CK10 in normal and benign laryngeal samples, while expression of CK17 was heterogeneous.[9] In contrast to the results of the current study, heterogeneity (25% to 75% staining by immunohistochemistry) of CK17 expression was also found in laryngeal SCC in relation to tumor grade.[22] Thus, to establish CK17 as a biochemical marker for laryngeal cancer, an additional study that consists of a larger number of cases of laryngeal cancer is required.

In contrast to other CKs, we found that expression of CK10 and CK17 was more pronounced in the cancerous lesions than in the normal samples (see Figure and Table 2). CK10 showed high sensitivity (90.9%), but only moderate specificity (69.2%), whereas CK17 showed both high sensitivity and specificity (91% and 92.3%, respectively; positive predictive value, 91%). Our results demonstrate that expression of these CKs is modified by malignant transformation; thus, they are potential markers for the detection of laryngeal cancer. Expression of either CK10 or CK17 did not correlate to the stage or grade of the tumor (Table 2). This may indicate an early stage in the carcinogenesis process, in which the epithelial cell shifts from the "normal set" of CK expression to a "malignant set" that includes CK10 and CK17.

To further examine the potential use of CK10 and CK17 as possible markers of laryngeal cancer, we took cancerous and normal samples from each of the 24 patients and tested them for an association between the presence of CKs and the occurrence of cancer. In this analysis, expression of CK10 in SCC was not significant, but expression of CK17 was significantly correlated to the presence of malignant transformation ($p = .016$). These results suggest that CK17 is a good biochemical marker for detection of

SCC of the larynx. Expression of CK17 has been studied in other tissues, such as urothelium,[23] and it has been suggested as a marker of tumor progression. Further investigation is needed to establish the expression of CK17 as a marker for the detection of laryngeal cancer. Moreover, the use of CK17 as a potential marker for detection and follow-up of patients with laryngeal cancer could be enhanced if its expression is found in samples of serum or sputum. This noninvasive approach would not only benefit patients, but also reduce costs for the health care system.

The use of other biochemical markers for the detection of laryngeal cancer has been suggested. Several studies have examined the expression of p53 in laryngeal cancer by immunohistochemistry and found increased expression of the protein in tumors, with a sensitivity varying between 61%[24,25] and 75%.[26] This overexpression of p53 correlated significantly with decreased survival.[27] The use of other markers, such as Ki-67, bcl-2, and proliferating cell nuclear antigen molecules, which are known as tumor markers, is controversial. Several studies show that staining for these molecules failed to detect laryngeal malignancy,[26,27] whereas others demonstrate a correlation between the percentage of positive cells and the grade and stage of the tumor.[28] It is, therefore, clear that the current markers for detection of laryngeal cancer lack sensitivity. Thus, the high sensitivity and specificity exhibited by CK17 in this study place it as a leading candidate for use as a marker of laryngeal SCC.

Most studies demonstrating expression of CKs in the larynx employed immunohistochemistry as the method of choice. This method reveals the identity of the cell producing the CK, and can thus distinguish between production of CKs by normal epithelium and by cancerous cells. However, it is regarded as a nonquantitative method with low sensitivity. We used RT-PCR to detect the expression of mRNA coding for CKs. This method is considered extremely sensitive, and it allowed us to screen for the expression of many CKs in a short time. However, we used RNA, which was extracted from many cells in the tissue, without the ability to identify the cell type. Thus, a positive signal could, theoretically, result from a single cell, and not necessarily represent the typical profile of the surrounding tissue. In addition, we have used RT-PCR only to establish a qualitative result, but this method could also be employed to quantify the amount of mRNA produced in the tissue. We have used RT-PCR primarily to screen for possible candidates that could serve as biochemical markers for laryngeal cancer. As our preliminary results indicate, CK17 may become such as candidate, and therefore, additional investigation, which will determine the identity of the cells producing CK17 in the tissue (normal versus cancerous) by an immunohistochemical approach, is warranted.

In summary, we have examined the profile of CK expression in both normal and malignant samples of the larynx. Of all the CKs examined, only CK17 exhibited both high sensitivity and specificity. Although no correlation was found between the stage and grade of the tumors and the expression of CK17, the ability to detect expression of CK17 by RT-PCR with such high sensitivity and specificity suggests that it may become a good marker for the detection and follow-up of laryngeal cancer.

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REVISION FUNCTIONAL ENDOSCOPIC SINUS SURGERY

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Functional endoscopic sinus surgery (FESS) has recently become a popular procedure for treating chronic sinusitis. However, there is a 2% to 24% rate of primary FESS failure. Revision functional endoscopic sinus surgery (RESS) is indicated for patients who remain symptomatic after primary FESS and optimal medical therapy have failed. A retrospective study was conducted on all patients who underwent RESS in our department between April 1988 and March 1998. During this period, FESS was performed 1,227 times to treat chronic sinusitis; among those procedures, 142 were RESS. Complications occurred in 14 RESS procedures (9.9%). After RESS, 65% patients had improved. In this study, we concluded that although RESS is more difficult than primary FESS because of distorted or missing anatomic landmarks, complications did not increase with RESS if meticulous surgical procedures were performed. However, the 65% improvement rate with RESS was lower than that of primary FESS.

KEY WORDS — endoscopy, reoperation, sinusitis.

INTRODUCTION

Functional endoscopic sinus surgery (FESS) has become a popular procedure for treating chronic sinusitis.[1] The success rates of FESS have been reported as 76% to 98% in the literature.[2] However, there is a 2% to 24% failure rate for primary FESS. Among patients who have undergone unsuccessful primary FESS, some eventually require a second FESS procedure, or revision functional endoscopic sinus surgery (RESS), for management of their sinusitis.[3]

Although the techniques and principles of RESS are essentially the same as those of primary FESS,[1] RESS can be more difficult to perform because of distorted anatomy, a tendency to bleed, and scarring.[4] Although the efficacy and safety of RESS for treating recalcitrant sinusitis have been reported to be comparable to those of primary FESS,[5] many factors can affect the outcome of RESS.[2] In this study, we report our experience with RESS.

MATERIALS AND METHODS

We have used FESS to treat chronic sinusitis in our department since April 1988. As of March 1998, we had performed FESS 1,227 times in 1,112 patients with chronic sinusitis. Among the 1,112 patients, 119 patients had undergone RESS 142 times in our department during this period. All patients with known systemic diseases were excluded from the study. The definition of RESS in this study is FESS performed for the second or a subsequent time in a patient with chronic sinusitis. We define primary FESS as FESS performed for the first time in a patient with chronic sinusitis.

The indication for RESS was the same as that for primary FESS. Primary FESS was indicated for patients in whom optimal medical treatment failed, and RESS was indicated for patients in whom both FESS and optimal postoperative medical treatment failed. The definition of "failure of medical treatment" was unsatisfactory results after repeated and appropriate antibiotic therapy with adequate auxiliary treatment. "Repeated and appropriate antibiotic therapy" for RESS meant that antibiotic management was given after surgery for at least 6 months, and the selection of antibiotics was based on clinical experience or on the results of bacterial cultures. According to the clinical course and findings for these patients, some auxiliary treatment was added. The auxiliary treatments included mucolytic agents, antihistamines, nasal steroids, and nasal douching. If patients remained symptomatic or if sinusitis recurred at least 4 times per year despite maximal and adequate medical treatment, RESS was indicated.

File review, computed tomography (CT), and questionnaires were used to analyze preoperative disease staging, operative procedures, complications, and the outcome of RESS. The outcome was classified as improved if signs or symptoms of the disease became better after FESS. When the signs or symptoms remained the same or became worse, the outcome was classified as unimproved.

RESULTS

We performed RESS 142 times to treat chronic sinusitis between April 1988 and March 1998 in our

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TABLE 1. STAGES OF CHRONIC SINUSITIS BEFORE PRIMARY FESS AND RESS

Staging	*Before Primary FESS**	*Before RESS†*
Kennedy staging system[6]		
Stage 0	2	
Stage I	1	1
Stage II	10	16
Stage III	24	47
Stage IV	8	8
Unclassified	2	6
Lund-Mackay staging system[6]		
Score 0	3	1
Score 1	3	1
Score 2	3	13
Score 3	5	7
Score 4	4	10
Score 5	8	13
Score 6	6	16
Score 7	21	32
Score 8	8	18
Score 9	4	8
Score 10	4	5
Score 11	1	7
Score 12	7	7

FESS — functional endoscopic sinus surgery, RESS — revision functional endoscopic sinus surgery.

*Preoperative computed tomography (CT) scans were available for 47 patients. Among them, there were 76 operative sides on which RESS was later performed.

†CT scans were available for 78 patients. Among them, there were 138 operative sides for RESS.

department, which accounted for 11.6% of the times we performed FESS. These 142 operations were performed in 119 patients. Of these patients, 85 underwent primary FESS and RESS in our department, and the remaining 34 had undergone primary FESS in other hospitals. Among them, 95 patients had undergone FESS twice (1 primary FESS and 1 RESS), 22 had undergone FESS 3 times, and 2 had undergone FESS 4 times. Their ages at the time of their first RESS procedure in our department ranged from 9 to 75 years, with a mean age of 39.5 years. Sixty-nine patients were male, and the other 50 were female.

During the same period, 1,078 patients underwent primary FESS 1,085 times for chronic sinusitis in our department. Among them, 7 patients underwent unilateral primary FESS at 2 different times. Their ages at their first primary FESS in our department ranged from 5 to 84 years, with a mean age of 40.3 years. Six hundred seventy-six patients were male, and the other 402 were female.

The indications for RESS were chronic sinusitis without polyps in 96 operations, nasal polyps in 45 operations, and antrochoanal polyps in 1 operation. In contrast, the indications for primary FESS were chronic sinusitis without polyps in 532 operations, nasal polyps in 517 operations, and antrochoanal polyps in 36 operations. The indications for FESS were significantly different between RESS and primary FESS for chronic sinusitis without polyps and nasal polyps ($p < .001$).

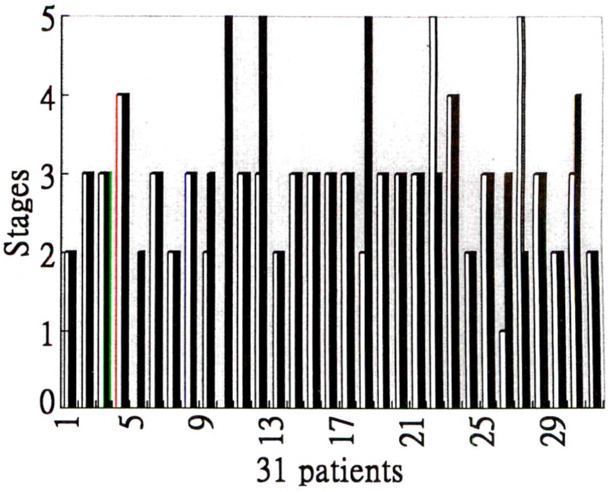

Fig 1. Comparison of pre–revision functional endoscopic sinus surgery (pre-RESS) stages (dark bars) with stages before previous functional endoscopic sinus surgery (FESS; light bars) by Kennedy staging system.[6] Stage 0 is coded as 0, stage I as 1, stage II as 2, stage III as 3, stage IV as 4, and unclassified stage as 5.

Computed tomographic scans performed before RESS were available for analysis for 78 operations (138 operative sides). The extent of disease before RESS was analyzed with the Kennedy staging system[6] and the Lund-Mackay staging system[6] (Table 1). According to the Lund-Mackay system, the maxillary sinus was clear on 13 sides, partially opacified on 94 sides, and totally opacified on 31 sides. The

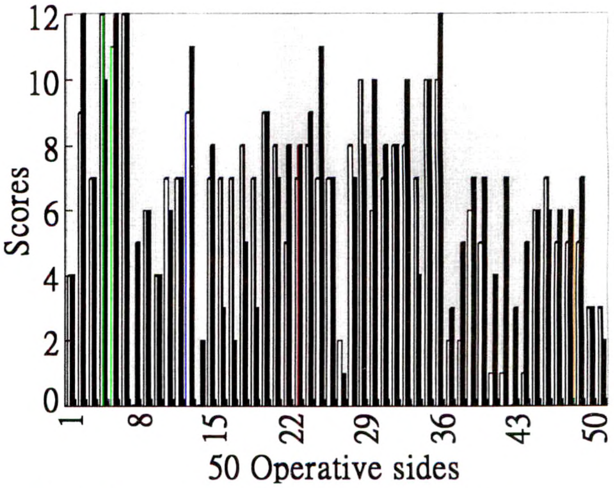

Fig 2. Comparison of pre-RESS stages (dark bars) with stages before previous FESS (light bars) by Lund-Mackay staging system.[6]

TABLE 2. OPERATIVE PROCEDURES USED IN 132 RESS PROCEDURES (220 OPERATIVE SIDES)

Procedures	*No.*	*%*
Anterior ethmoidectomy	189	85.9
Posterior ethmoidectomy	185	84.1
Maxillary antrostomy	173	78.6
Frontal sinusotomy	158	71.8
Sphenoidotomy	123	55.9

anterior ethmoid sinus was clear on 9 sides, partially opacified on 99 sides, and totally opacified on 30 sides. The posterior ethmoid sinus was clear on 33 sides, partially opacified on 84 sides, and totally opacified on 21 sides. The sphenoid sinus was clear on 62 sides, partially opacified on 54 sides, and totally opacified on 22 sides. The frontal sinus was clear on 35 sides, partially opacified on 54 sides, and totally opacified on 49 sides. The ostiomeatal complex was open on 33 sides and occluded on 105 sides. Additionally, CT scans performed before primary FESS were available for analysis in 47 cases (76 operative sides; Table 1). Furthermore, the extent of disease existing before RESS and that existing before the previous FESS could be compared in 31 cases (Figs 1[6] and 2[6]).

We performed RESS 142 times: 112 operations were bilateral, and 30 were unilateral. Forty-eight operations were performed with general anesthesia, and 94 operations with local anesthesia. The operative procedures could be analyzed in 132 RESS procedures (220 operative sides; Table 2). Among them, operative procedures could be compared between primary FESS and RESS on 146 operative sides. A greater number of operative procedures were done on 30 RESS sides. Fifty-seven RESS sides underwent the same number of operative procedures as primary FESS. Fewer operative procedures were performed on 59 RESS sides.

Operative complications occurred in 14 RESS procedures (Table 3). The operative complication rate was 9.9%. In comparison, 107 operative complications occurred in the 1,085 primary FESS procedures (9.9%). Major operative complications occurred in 3 RESS procedures. The nasolacrimal duct was injured in 1 patient, and blood transfusion was required for another 2 patients.

The outcome of RESS was evaluated with questionnaires. Sixty patients responded effectively to the questionnaires. The follow-up period ranged from 8 months to 9 years 6 months, with a mean of 3 years. Among patients with chronic sinusitis without polyps, 28 indicated that the symptoms had improved, 12 considered the symptoms to be the same as before surgery, and 5 complained that the symptoms had worsened. Among patients with nasal polyps, 1 reported no symptoms at all, 10 indicated that the symptoms had improved, 2 considered the symptoms to be the same as before surgery, and 2 complained that the symptoms had worsened. The overall improvement rate was 65%. In contrast, 487 patients who underwent only primary FESS responded effectively to the questionnaires. The follow-up period ranged from 7 months to 10 years 6 months, with a mean period of 3 years 9 months. Among patients with chronic sinusitis without polyps, 29 reported no symptoms at all, 145 indicated that the symptoms had improved, 47 considered the symptoms to be the same as before surgery, and 17 complained that the symptoms had worsened. Among patients with nasal polyps, 32 reported no symptoms at all, 164 indicated that the symptoms had improved, 28 considered the symptoms to be the same as before surgery, and 8 complained that the symptoms had worsened. Among patients with antrochoanal polyps, 3 reported no symptoms at all, 13 indicated that the symptoms had improved, and 1 complained that the symptoms had worsened. The overall improvement rate in patients who underwent only primary FESS was 79.3% (386 of 487 patients). The difference was statistically significant (p = .013).

TABLE 3. NUMBERS OF OPERATIVE COMPLICATIONS

Complications	*RESS*	*Primary FESS*
Orbital fat extrusion	5	32
Epistaxis	4	22
Need for transfusion	2	25
Dura exposure	1	1
Periorbita exposure	1	6
Nasolacrimal duct injury	1	5
Stopping FESS because of pain	0	8
Diplopia	0	3
Cerebrospinal fluid leakage	0	3
Left nasal packs	0	1
Atrophic rhinitis	0	1
Total	14	107

DISCUSSION

Although FESS has become a standard procedure for treating chronic sinusitis, there is a 2% to 24% failure rate for primary FESS.[2] For patients who have undergone unsuccessful primary FESS, RESS has been reported to be effective.[7] In general, RESS is needed after 10% to 18% of primary procedures.[3] In this study, RESS accounted for 11.6% of our operations. Therefore, RESS was performed quite commonly. As compared with indications for primary FESS, chronic sinusitis without polyps was a significantly more frequent indication for RESS. In contrast, nasal polyps were a significantly more frequent indication for primary FESS than for RESS. Thus, patients who underwent RESS in this study did so more commonly because of persistent sinusitis symptoms after FESS rather than for residual or recurrent polyps.

Failure of primary FESS can have many different causes, including systemic disorders, anatomic abnormalities, surgical techniques, and management deficiencies.[3,8] However, the extent of disease has been considered as the only relevant factor useful in predicting FESS failure.[2] When the extent of disease was analyzed with the Kennedy staging system[6] and the Lund-Mackay staging system[6] on the basis of CT findings, more than half of the patients who underwent RESS in this study were considered to have advanced disease (defined as stages III and IV by the Kennedy system or as a score greater than 6 by the Lund-Mackay system) both before primary FESS and before RESS. Ramadan[8] also found that patients who underwent RESS had had a higher mean score (Lund-Mackay system) before primary FESS than did those who underwent successful FESS. On the other hand, no other RESS study has compared the extent of disease before primary FESS with the extent of disease before RESS. In this study, most patients had similar or more advanced disease before RESS as compared to before the previous FESS. Hence, a failure to decrease the extent of the disease by primary FESS might be another important factor in RESS, in addition to the factor of more advanced disease before primary FESS. However, no definite conclusion could be drawn, because of lack of a control group.

The operative procedures for RESS were basically similar to those for primary FESS.[1] However, RESS was considered to be more difficult, because of missing anatomic landmarks, increased bleeding, adhesions, and less experience of the surgeons with RESS than with primary FESS.[2,4] Usually, we used the remnant of the middle turbinate, sphenoid ostium, medial orbital wall, and skull base as surgical landmarks. These were also suggested as useful landmarks in other RESS studies.[2,4] In addition to surgical landmarks, computer-aided image guidance systems have been used to help identify anatomic structures during surgery, especially for revision cases, although they cannot substitute for thorough anatomic and surgical training.[9]

Ramadan[8] reported that residual air cells and maxillary sinus ostium stenosis were the most common operative findings of his RESS procedures. On the other hand, disease existed in the majority of maxillary, ethmoid, and sphenoid sinuses in the RESS patients of King et al.[5] Therefore, maxillary antrostomy was performed in all of their patients, 88.4% of their patients underwent ethmoidectomy, 48.8% of their patients underwent sphenoidotomy, and only 37.2% of their patients underwent a frontal sinusotomy.[5] Among our RESS patients, ethmoidectomy was the most common operative procedure, followed by maxillary antrostomy. Therefore, residual air cells and maxillary sinus ostium stenosis were the most common reasons for our RESS procedures. As compared with the operative procedures of primary FESS, more procedures needed to be done in 21% of RESS procedures. This finding indicated that in addition to residual disease, recurrent disease was one cause of our RESS.

Performing RESS is more difficult than performing primary FESS, and the complication rates for RESS have been suggested to be higher than those for primary FESS.[2] However, no major complications occurred in the RESS patients of King et al.[5] In other studies, complication rates of RESS were reported as being from 1.1% to 12.5%.[8] In our study, operative complications occurred in RESS as frequently as in primary FESS (9.9%). Major complications occurred in only 3 of 142 RESS procedures. Intimate knowledge of sinus anatomy, experience with the operative techniques, and careful preoperative and intraoperative preparation help reduce the complications of RESS.[5] Computer-aided image guidance systems also help reduce operative complications in difficult and revision cases, although the complication rate of FESS might not be reduced by use of computer-aided image guidance systems.[9]

The reported success rates of RESS range from 50% to 90.9% in the literature.[2] Lazar et al[1] and King et al[5] considered their results for RESS to be comparable to those for primary FESS. However, no study has actually compared the results of RESS with those of primary FESS. Our 65% success rate for RESS procedures is comparable to the success rates of 2 recent studies: 69.8% in the series of King et al[5] and 66.7% in that of Moses et al.[2] However, the 65% success rate for RESS is lower than the 79.3% success rate for our primary FESS procedures (p = .013). Mucopurulent rhinorrhea and nasal polyps were the 2 major reasons for RESS, and mucopurulent rhinorrhea was the most common symptom relieved by RESS. In contrast, postnasal discharge was the most common symptom remaining after RESS. Besides surgical technique, aggressive postoperative care and follow-up may be necessary to reduce the failure rate of RESS.[2,4]

CONCLUSION

Because primary FESS has a 2% to 24% failure rate, RESS frequently needs to be performed to treat patients who have undergone unsuccessful primary FESS. It is a more difficult procedure than primary FESS, because of distorted or missing anatomic landmarks. However, RESS can be relatively safely accomplished if meticulous surgical procedures are performed. A 65% success rate was achieved for RESS in this study; still, this was lower than the success rate for primary FESS. Improved surgical technique and aggressive postoperative care may be necessary to reduce the failure rate of RESS.

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CLINICAL STUDY OF ACUTE LOW-TONE SENSORINEURAL HEARING LOSS: SURVEY AND ANALYSIS OF GLYCEROL TEST AND ORTHOSTATIC TEST

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Fifty patients (14 men and 36 women ranging in age from 16 to 66 years) with acute low-tone sensorineural hearing loss (ALHL) were retrospectively analyzed in this study. The glycerol test and the orthostatic test were performed. On the glycerol test, 43.8% of 16 ears of the 14 men and 33.3% of 39 ears of the 36 women with ALHL had a positive result. On the orthostatic test, 42.9% of the 14 men and 52.7% of the 36 women with ALHL had a positive result. In addition, 28.6% of the 14 men and 33.3% of the 36 women had hypotension with a systolic blood pressure of 100 mm Hg or less. Our results suggest that endolymphatic hydrops as shown by the glycerol test and an underlying autonomic imbalance and/or insufficient blood circulation as shown by the orthostatic test may be important factors in the causation of ALHL.

KEY WORDS — acute low-tone hearing loss, endolymphatic hydrops, glycerol test, orthostatic test.

INTRODUCTION

Individuals with acute-onset and slight low-tone hearing loss are frequently identified at outpatient clinics. However, this disease is not always given close attention, since the hearing loss sometimes recovers spontaneously and the prognosis is relatively good. In addition, these individuals do not always complain of hearing loss at first and often complain instead of a sensation of ear fullness, tinnitus, or autophony. Such ear symptoms are often not severe. It is reasonable to suspect, therefore, that some patients have been overlooked or treated for an obstruction of the eustachian tube as outpatients without undergoing any kind of hearing test. The first detailed clinical review of patients with low-tone sensorineural hearing loss was reported by Abe[1] in 1982. Acute low-tone sensorineural hearing loss (ALHL) became a main topic of discussion at the 38th Japan Audiology Congress in 1993. However, it seems that this disease entity is not being discussed widely, except in Japan. There have been some reports,[1-6] including ours,[6] on ALHL, but the criteria of ALHL have still not been defined as yet, and the cause of the disease is not clear. The purpose of the present study was to investigate the clinical features and the pathogenesis of ALHL.

PATIENTS AND METHODS

Fifty patients (14 men and 36 women) who were seen at Yamanashi Medical University with otologic symptoms such as hearing loss, tinnitus, and a sensation of ear fullness were selected for study. Our diagnostic criteria for ALHL are as follows: 1) the hearing loss is purely sensorineural in nature, and tympanometry (model RS20, RION, Tokyo, Japan) shows normal type A and normal tympanic membrane findings; 2) on a pure tone audiogram (model AA61BN, RION), the average hearing threshold at the 3 low frequencies (125, 250, and 500 Hz) is 30 dB or more, and the average hearing threshold at the 3 higher frequencies (2, 4, and 8 kHz) is 20 dB or less; and 3) the patient has no known etiologic factors that might cause low-tone sensorineural hearing loss as detected by routine clinical and radiologic examinations. Patients with a history of episodic vertigo and/or dizziness and with spontaneous nystagmus at the initial examination were strictly excluded from this study. The details of ALHL and some routine tests for ALHL have been described previously.[6]

A glycerol test was performed for each patient by the intravenous administration of 10% glycerol (500 mL) for 2 hours instead of oral administration. Pure tone audiometry was performed immediately after the glycerol administration and 1 and 2 hours later. The criterion used for a positive test result was that of at least 10 dB improvement in pure tone threshold at 2 or more frequencies, as proposed by Aso et al.[7]

The orthostatic test (active standing test) was ad-

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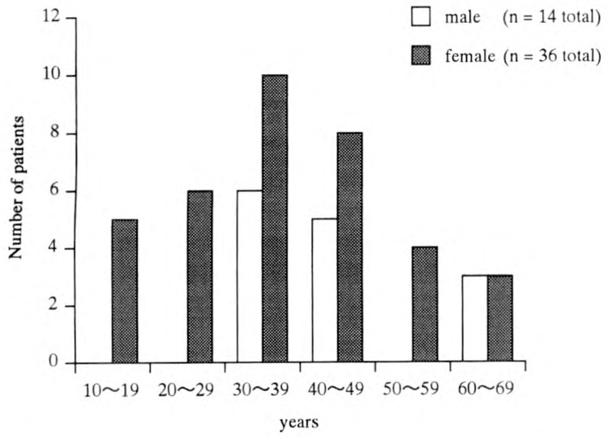

Fig 1. Age distribution of 50 patients with acute low-tone sensorineural hearing loss (ALHL). In both male and female patients, peak incidence was in thirties.

ministered to patients with ALHL according to a standardized protocol. In brief, after the patients had remained for 10 minutes in the supine position, the blood pressure and pulse rate at rest were measured. These measurements were repeated 1, 5, and 10 minutes after the subjects rose to the standing position. A positive orthostatic test result was defined as a systolic pressure decrease of more than 21 mm Hg, a pulse pressure decrease of more than 16 mm Hg, or a pulse rate increase of more than 21 beats per minute at any of the 3 measurement periods in the standing position. Age- and sex-matched controls (48 men and 60 women) were chosen randomly from a group of volunteers who had no history of hearing impairment or vertigo. The χ^2 test was used for statistical analysis of the data, and a p value of less than .05 was regarded as significant.

RESULTS

Patient Status. There were 14 men with a mean age of 44.6 years and 36 women with a mean age of 36.7 years. The peak incidence by age in both was the fourth decade of life (Fig 1). There were 22 cases in the right ear, 23 cases in the left ear, and 5 bilateral cases (see Table).

Clinical Symptoms. Tinnitus and a sensation of ear fullness were the most frequent clinical symptoms (61.8% each), followed by hearing loss (52.7%) and slight unsteadiness and/or dizzy sensation (33.3%; Fig 2).

SUBJECTS

	Hearing Loss			
	Right	*Left*	*Bilateral*	*No. of Ears*
Male	7	5	2	16
Female	15	18	3	39
Total	22	23	5	55

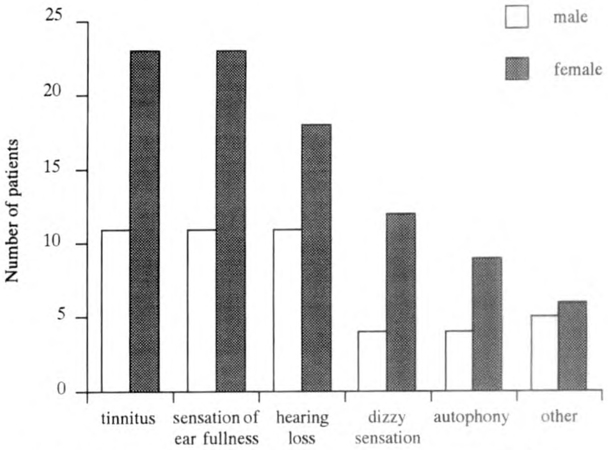

Fig 2. Incidence of chief clinical symptoms of 50 patients with ALHL.

Audiometric Pattern. The mean hearing threshold at the 3 low frequencies in the affected ears was 42.2 dB in the male patients and 41.8 dB in the female patients. The mean hearing threshold at the 3 low frequencies in the unaffected ears was 17.8 dB in the men and 17.3 dB in the women (Fig 3).

Glycerol Test. The changes in hearing level (mean value ± 1 SD) of the 55 ears with ALHL after the glycerol test are summarized in Fig 4. In the male patients, the average hearing improvement was 5.9 dB at 125 Hz, 7.5 dB at 250 Hz, and 6.3 dB at 500 Hz, and the total hearing improvement was 19.7 dB at the 3 low frequencies. In the female patients, the average hearing improvement was 6.8 dB at 125 Hz, 6.4 dB at 250 Hz, and 5.5 dB at 500 Hz, and the total hearing improvement was 18.7 dB at the 3 low frequencies. On the glycerol test, 43.8% of the 16 affected ears of the 14 men with ALHL and 33.3% of the 39 affected ears of the 36 women with ALHL had a positive result.

Orthostatic Test. On the orthostatic test, 42.9% of the men and 52.7% of the women with ALHL had a positive result. That is, 50% of the patients had a positive result, while only 26.9% of the control group did ($p < .05$).

Of the 14 men tested, 28.6% showed hypotension with a systolic blood pressure of 100 mm Hg or less. The systolic blood pressure in 78.6% of these 14 men was lower than that in the normal male population in Japan (Fig 5A).[8] Of the 36 women tested, 33.3% showed hypotension. The systolic blood pressure in 80.6% of these 36 women was lower than that of the normal female population in the source cited above (Fig 5B).[8]

DISCUSSION

Acute low-tone sensorineural hearing loss became a main topic of discussion at the 38th Japan Audiology Congress in 1993. However, the concept of this

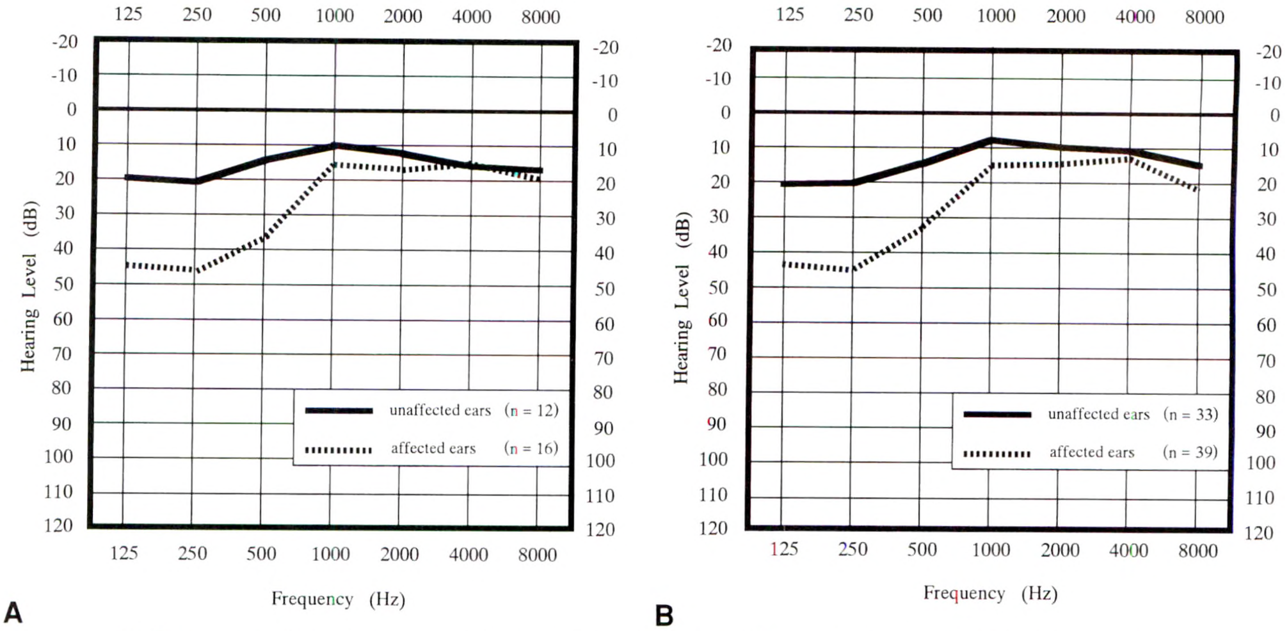

Fig 3. Average values of pure tone audiograms of **A**) 14 male patients and **B**) 36 female patients with ALHL.

disease is not widespread outside of Japan, and it is usually ignored in current leading medical textbooks, since such hearing loss sometimes recovers spontaneously and the prognosis is relatively good. It might be considered a "non-disease" by some, since the patients' complaints generally do not indicate severity. However, the number of patients with ALHL is gradually increasing in our outpatient clinic. Thus, it is thought that clarification of the cause of ALHL is very important.

It is known that low-tone sensorineural hearing loss is one of the most important audiographic findings in Meniere's disease; its cause is at present described as endolymphatic hydrops. Glycerol ingestion produces hearing improvement as a result of the reduction of endolymphatic hydrops through "osmotic" diuresis. We used an intravenous administration of 10% glycerol for the test instead of oral administration, because oral administration sometimes produces side effects such as nausea, vomiting, and severe headache. Aso et al[7] concluded that the intravenously administered glycerol test is a highly useful method for the detection of endolymphatic hydrops and has no severe side effects. In the present study, on the glycerol test, 43.8% of the men and 33.3% of the women with ALHL had a positive result. A positive result on the glycerol test suggests that a patient

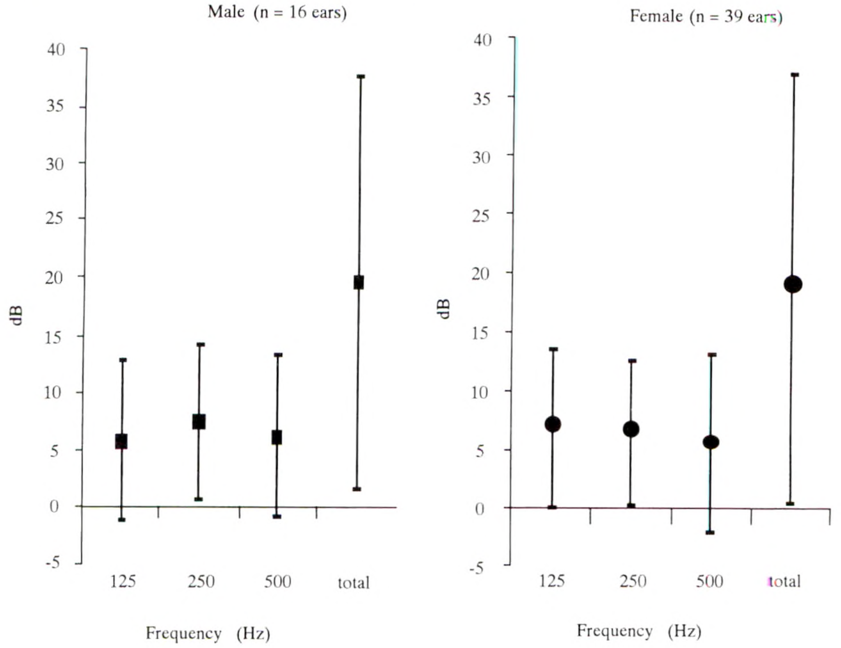

Fig 4. Changes in hearing level in 55 ears with ALHL after glycerol test. Symbols and bars indicate mean ± SD.

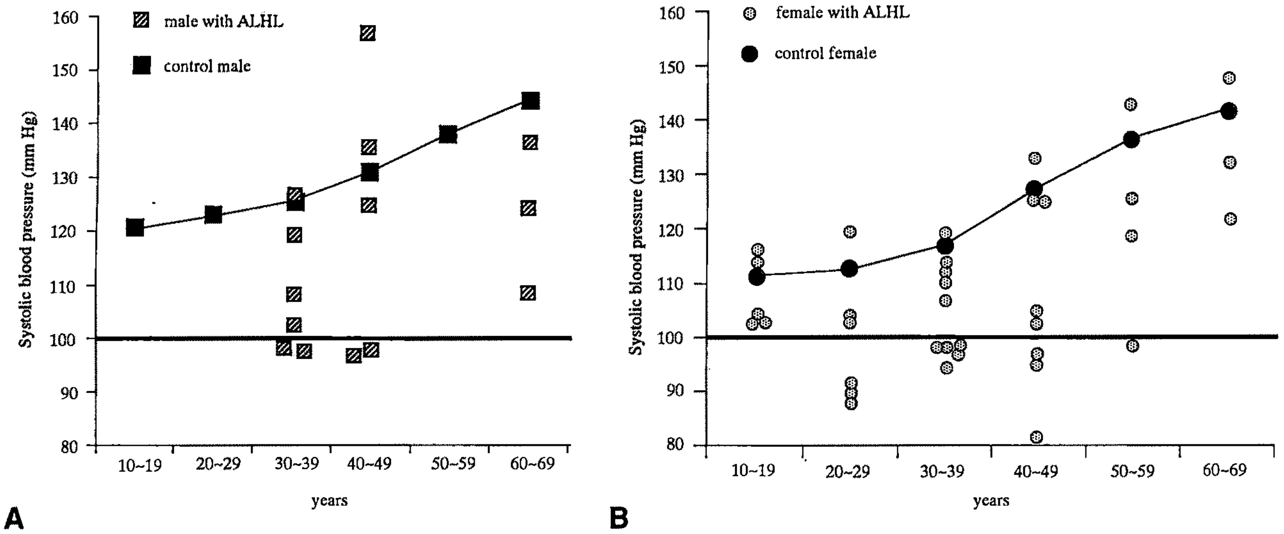

Fig 5. Systolic blood pressure distribution of **A)** 14 male patients and **B)** 36 female patients with ALHL. Systolic blood pressure in 78.6% of male patients was lower than that of normal male population; it was lower in 80.6% of female patients than that of normal female population.[8]

has endolymphatic hydrops or Meniere's disease. Yamasoba et al[3] examined 24 ALHL patients using electrocochleography. An abnormal increase in the negative summating potential amplitude following click stimuli was found in 54% of these patients, and the negative summating potential–action potential ratio was abnormally increased in 63% of the patients. Yamasoba et al concluded that the pathophysiology of ALHL may be similar to that of endolymphatic hydrops, and that ALHL does not always lead to cochlear or classic Meniere's disease. On the glycerol test in our study, the average total hearing improvements were 19.7 dB and 18.7 dB at the 3 low frequencies in the male and the female patients, respectively. It is suggested that endolymphatic hydrops is mainly involved in the causation of ALHL.

Isosorbide has been used to treat Meniere's disease, but is not always effective for ALHL patients who show positive glycerol test results. In this study, about 80% of patients with ALHL showed complete or partial recovery and did not experience fluctuating hearing, and only 2 of the 50 patients received diagnoses of classic Meniere's disease within the 3 years of follow-up. It is thought that the pathophysiology of ALHL may be similar to that of endolymphatic hydrops, but endolymphatic hydrops alone does not fully explain the mechanism underlying ALHL.

In 1950, Williams et al[9] suggested that the criteria of endolymphatic hydrops without vertigo were as follows: 1) a hearing loss for the low tones, with high tones more or less preserved; 2) a history of fluctuating hearing loss (in women, a temporary loss associated with menses); 3) certain allergic phenomena occurring in association with the impairment of hearing (vasomotor rhinitis, urticaria, dermatitis, severe unilateral headaches); and 4) a sudden decrease in the ability to hear, occurring within 2 or 3 days, with persistence of the impairment. Our analysis of clinical records revealed the following characteristics of ALHL: female preponderance; peak incidence during the fourth decade of life; frequent accompanying tinnitus, sensation of ear fullness, and/or autophony; and association with autonomic imbalance. Allergic symptoms and temporary hearing loss associated with menses were not evident in our cases, nor in other cases of ALHL in Japan.[1-6] Thus, the present results suggest that the criteria of endolymphatic hydrops without vertigo and the criteria of ALHL in Japanese patients may differ slightly.

In the present study, the ALHL patients frequently had positive results on the Schellong (orthostatic) test, which is a test of autonomic nervous system function. This test is useful for investigating the relationship between orthostatic changes in blood pressure and pulse rate. There are several reports[10-13] on the orthostatic test relative to dizziness and vertigo. In our study, 50% of patients had a positive result. In addition, hypotension was found in about one third of the patients, and three fourths of them showed lower blood pressure than the large Japanese control population.[8] This finding suggests that patients with ALHL have an abnormal systemic condition related to an underlying autonomic imbalance and/or insufficient blood circulation. In some of the patients in this study, medication and/or physical treatment for hypotension or autonomic imbalance resulted in improvement in that condition and in hearing — a finding suggesting some transient or reversible circulatory disturbance in the cochlea caused by autonomic nerve dysfunction. A clinical study[14] using ultrasonic rheography revealed that vertiginous patients with hypotension and/or a positive orthostatic hypotension test result have insufficient vertebral blood flow. Ito et al[14] pointed out that low-frequency hearing loss is an important audiometric indication of vertebrobasilar insufficiency. They reported that dizzy patients with hypotension had low-tone hearing loss and that their hearing symptoms improved when an antihypotensive drug was administered.[15] Pirodda et al[16] investigated the possible effect of systemic arterial pressure in a retrospective study of patients no more than 40 years old who were treated for sudden hearing loss, comparing the mean values of their arterial pressure with those of a control group of 25 subjects of similar age who were admitted for other disorders. The significantly lower mean values of arterial pressure in the group affected by sudden hearing loss and the easier reversibility of the damage in these patients suggest that at least in some cases, the cochlear damage may be caused by a perfusion deficit due to the combined effect of hypotension and imperfect vasomotor regulation. The present patients with ALHL did not complain of vertigo, but one third reported some light-headedness, which may have been related to hypotension. In some of our present patients, medication for hypotension brought about an improvement in their condition and hearing. However, in other patients, medication for hypotension was not effective. This result indicates that hypotension alone cannot explain the mechanism of ALHL.

In light of the present results, we propose that endolymphatic hydrops as shown by the glycerol test and an underlying autonomic imbalance and/or insufficient blood circulation as shown by the orthostatic test are mainly involved in the causation of ALHL. Needless to say, these two factors may not explain the complete mechanism of ALHL. We believe that if more physicians pay attention to cases of slight hearing loss accompanied by mild ear symptoms such as tinnitus, a sensation of ear fullness, or autophony, the cause of ALHL will be clarified in the near future.

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ACUTE TONSILLITIS AS THE FIRST MANIFESTATION OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER

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Post-transplant lymphoproliferative disorder (PTLD) is a life-threatening complication that may follow orthotopic liver transplantation (OLT) in children. The first symptoms are often in the ear, nose, or throat (ENT) area. This abnormal proliferation of lymphoid cells is related to Epstein-Barr virus (EBV) infection in immunocompromised children. The incidence of PTLD, EBV status before OLT and at the diagnosis of PTLD, delay between OLT and PTLD, localization, pathological investigations, and the treatment and evolution of PTLD were prospectively evaluated in 77 pediatric liver transplant recipients. Eight patients (10%) developed PTLD, all with an ENT presentation. Seven had acute nonbacterial tonsillitis (with a negative throat swab), and 1 had a pharyngolaryngeal localization at the time of the diagnosis. Four patients had associated involvement outside the ENT area. All patients were EBV-seronegative at the time of OLT; 6 underwent seroconversion at the time of diagnosis, and 2 within 9 and 20 months of diagnosis. All patients presented with low-grade PTLD. All patients with acute tonsillitis associated with EBV seroconversion underwent immediate tonsillectomy, and immunosuppression was decreased as much as tolerated. This therapeutic protocol led to complete recovery in all patients. After OLT in children, nonbacterial tonsillar inflammation or hypertrophy associated with an EBV infection is often the first manifestation of PTLD. Tonsillectomy combined with tapering of immunosuppression offers the best chance for a complete recovery.

KEY WORDS — child, Epstein-Barr virus, liver transplantation, lymphoma, malignancy, post-transplant lymphoproliferative disorder, tonsillitis.

INTRODUCTION

After liver transplant, children are at risk of developing B cell lymphoproliferation. This well-known complication, named post-transplant lymphoproliferative disorder (PTLD), is responsible for a significant level of mortality. It is a histologically and genetically heterogeneous disorder of which the hallmark is the abnormal proliferation of lymphoid cells, ranging from reactive polyclonal lymphoid hyperplasia to monoclonal malignant lymphoma.[1,2] The development of PTLD is closely associated with Epstein-Barr virus (EBV) infection,[3-5] which induces B cell proliferation not regulated by T lymphocytes in immunosuppressed individuals.[6-8] We present a 3-year prospective study of PTLD following pediatric orthotopic liver transplantation (OLT) performed at Saint-Luc Hospital, Brussels, Belgium, showing that tonsillitis is frequently the first sign of PTLD, and emphasizing the therapeutic role of tonsillectomy combined with reduction of immunosuppression.

MATERIAL AND METHODS

We prospectively evaluated all patients who underwent OLT between July 1996 and June 1999 for clinical signs of PTLD, including a systematic ear, nose, and throat (ENT) examination. The data collected included gender, age at the time of transplantation, reason for the transplantation, EBV status before OLT and at the time of PTLD, delay between transplantation and PTLD, and localization, pathological classification, treatment, and evolution of PTLD.

Post-transplant immunosuppressive agents included cyclosporine (Sandimmun, Novartis, Bale, Switzerland), prednisolone, and azathioprine (Imuran, Glaxo Wellcome, Brussels). In the most recent cases, tacrolimus (Prograft, Fujisawa Corporation, Berlin, Germany) was used instead of cyclosporine. Rejection episodes were treated with high doses of intravenous methylprednisolone. In cases of steroid-resistant rejection in patients taking cyclosporine, this drug was replaced by tacrolimus.[3,4] Serum anti-EBV IgG and IgM levels were obtained before OLT, every 2 weeks during the first 3 months after OLT, and then monthly for 9 months. A polymerase chain reaction test to detect EBV genome viral sequences in circulating lymphocytes was performed before OLT, monthly for 3 months after OLT, and then at 6 and 12 months. The tests were also performed when PTLD was suspected clinically. We suspected PTLD in the

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RESULTS

Patient No.	*OLT No. for Institution*	*Age at OLT (mo)*	*Localization of PTLD*	*Pathological Classification*	*OLT to PTLD (mo)*	*EBV Before OLT*	*EBV at Time of PTLD*	*LMP1 in Tonsils*	*Treatment*	*Evolution*
1	880	47	Tonsils	Mononucleosis-like	2	–	Seroconversion	+	Tonsillectomy, reduction of immunosuppression	Complete remission, dead of other cause
2	905	17	Tonsils, liver, stomach, colon, enteric lymph nodes	Polymorphic	3	–	Seroconversion	+	Tonsillectomy, immunosuppression discontinued	Complete remission
3	953	6	Tonsils, stomach	Polymorphic	12	–	+ at 3 mo after OLT	+	Tonsillectomy, immunosuppression discontinued	Complete remission
4	974	7	Tonsils	Polymorphic	1	–	Seroconversion	+	Tonsillectomy, reduction of immunosuppression	Complete remission
5	988	28	Tonsils, liver	Mononucleosis-like	2	–	Seroconversion	+	Tonsillectomy, immunosuppression discontinued, anti-CD20 antibodies	Complete remission, second OLT
6	1,000	10	Tonsils, stomach, duodenum	Polymorphic	5	–	Seroconversion	+	Tonsillectomy, immunosuppression discontinued	Complete remission
7	1,021	8	Tonsils	Polymorphic	2	–	Seroconversion	+	Tonsillectomy, reduction of immunosuppression	Complete remission
8	907	12	Pharynx, larynx	Polymorphic	23	–	+ at 3 mo after OLT	+	Immunosuppression discontinued	Complete remission

OLT — orthotopic liver transplantation, PTLD — post-transplant lymphoproliferative disorder, EBV — Epstein-Barr virus, LMP1 — latent membrane protein–1.

following situations: acute nonbacterial tonsillitis (bacterial infection excluded by a throat swab), fever, worsening of general condition, loss of appetite, weight loss, irritability, gastrointestinal symptoms such as diarrhea, bleeding, or abdominal pain, and splenic and/or lymph node enlargement. Any clinical suspicion led to further biochemical, virologic, imaging, and/or histologic investigations.

Biochemical investigations included assessment of hypergammaglobulinemia, assessment of monoclonal or oligoclonal IgG or IgM production, and absolute B lymphocyte count. Bone marrow aspiration was performed in case of an abnormal peripheral blood cell count (neutropenia or thrombocytopenia). Diagnosis of primary EBV infection, as previously described, included detection of IgM anti-EBV and/or detection of EBV genome by polymerase chain reaction. Solid tumors or lymph nodes were submitted to biopsy whenever possible for histologic analysis.[3,4] Immunostaining was performed for EBV latent membrane protein–1 (LMP1) and lymphocyte B CD20 marker. Tonsillectomy was systematically performed for diagnosis and therapy in all cases of acute tonsillitis without a demonstrated bacterial cause. Only patients with pathologically and immunologically demonstrated B lymphocyte proliferation were considered to have PTLD. We classified PTLD according to the recommendations of the Society for Hematopathology Workshop published in 1997.[9]

Single tonsil localization was treated by tonsillectomy associated with a reduction in immunosuppression as far as tolerated by the patient. The immunosuppression was discontinued in cases of extratonsillar involvement, if tolerated. One patient with no tumor regression was given treatment with anti-CD20 antibodies as described in the literature (Mabthera, Roche, Brussels).[3,4,10]

The patients were investigated daily for liver function, and the size of the tumors was followed with appropriate imaging techniques. Liver biopsy to detect rejection was performed whenever an increase in transaminases was observed.

Complete remission was established when all signs of lymphoproliferation had disappeared and liver function tests showed normal findings. Remission was considered partial whenever persistent lymphoid masses were detected.

RESULTS

Between July 1996 and June 1999, 77 patients underwent 84 OLTs. The mean age at the time of OLT was 2.8 years (range, 3 months to 13.4 years). The original diagnoses leading to transplantation included biliary atresia (n = 47), primary familial intrahepatic cholestasis (n = 9), Alagille syndrome (n = 3), cryptogenic cirrhosis (n = 5), fulminant hepatitis (n = 4), metabolic diseases (n = 4), liver tumors (n = 2), and miscellaneous other diagnoses (n = 3). Seven patients underwent re-transplantation for rejection (n = 4) or surgical complications (n = 3). Of the 77 patients, 8 developed PTLD (see Table). In all cases, the first symptoms leading to the diagnosis of PTLD were localized to the ENT area. Seven patients presented with acute nonbacterial tonsillitis detected during routine examination. Four of the 7 had associated involvement of extratonsillar sites: liver nodules (n = 2), stomach wall infiltration (n = 3), duodenal wall (n = 1), and mesenteric lymph nodes (n = 1). The mean time from OLT to the development of PTLD was 4 months. The PTLD was classified as mononucleosis-like in 5 patients and as polymorphic in 2.[9] All patients were seronegative at the time of OLT. We detected EBV IgM antibodies at the time of PTLD diagnosis in all but 1 patient, in whom they were detected within weeks of diagnosis. We detected EBV LMP1 in the tonsils by immunostaining in all patients. Complete remission of the PTLD was achieved in all patients after tonsillectomy and reduction (n = 3) or withdrawal of immunosuppression (n = 4). One patient developed chronic symptoms of rejection and required re-transplantation. One patient died of a cause unrelated to PTLD while in remission. The last patient presented a disseminated pharyngolaryngeal infiltration with no extra-ENT localization 23 months after OLT. Surgical excision was not possible, but the child responded well to discontinuation of immunosuppression and is currently in complete remission.

DISCUSSION

In this series of 8 patients with PTLD, the tonsils were the first site of disease, in keeping with the finding that tonsillar involvement is the most common early localization of this syndrome in the first months following OLT. Of the 8 patients, 7 had acute nonbacterial tonsillitis, and 1 had pharyngolaryngeal localization. In 4 cases, the ENT symptoms led to the detection of associated intra-abdominal lymphoid masses localized in the liver graft, the stomach, the duodenum, the intestine, and/or the mesenteric lymph nodes. This incidence of Waldeyer's ring involvement (87% of our PTLD cases) was higher than incidences previously reported,[11-16] perhaps because of our systematic detection protocol and the implementation of tonsillectomy in cases of tonsillar inflammation and/or enlargement occurring in the context of post-transplant EBV infection with a negative throat swab for bacterial infection. The overall PTLD incidence of 10% is within the 3% to 20% incidence reported in the pediatric age group.[3,4,16] It is noteworthy that our patients with PTLD had a mean age of 17 months, as compared to the global average age at OLT of 2.8 years. The higher incidence of EBV-negative status in younger patients probably explains the higher susceptibility to primary EBV infection, as the main risk factor for the development of PTLD is an EBV-negative recipient and an EBV-positive donor. All patients underwent immediate tonsillectomy, while immunosuppression decreased as much as was tolerated. This reduction of immunosuppression was followed by a rapid improvement of the patient's general condition, disappearance of fever, and regression of extratonsillar lymphoproliferative masses. Full recovery was obtained in all patients. One patient died of a cause unrelated to PTLD.

All patients presented, at the time of diagnosis, with a low-grade PTLD that was polymorphic and polyclonal. The lymphotropic DNA of herpes virus has been shown to replicate in oropharyngeal cells[17]; thus, tonsillar involvement is likely to be the first step in an EBV-associated lymphoproliferation, as it was in the present series.

We conclude that the first manifestation of PTLD is most frequently observed in the ENT area. Otolaryngologists should be aware of this complication and have a high index of suspicion in this high-risk population. Tonsil biopsy or tonsillectomy with lymphocyte subtyping is necessary to confirm the diagnosis. Early recognition of PTLD and therapeutic tonsillectomy, combined with immunosuppression reduction, may avoid dissemination of the disease.

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FUNCTIONAL NECK DISSECTION FOR THE CLINICALLY NEGATIVE NECK: EFFECTIVENESS AND CONTROVERSIES

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This study was performed to evaluate the effectiveness of functional neck dissection in controlling metastasis to the clinically negative (cN0) neck, focusing on recurrences in the pathologically negative (pN0) neck and the role of extracapsular spread in the cN0 neck. A series of 172 patients (253 dissected fields) treated for cN0 laryngeal or hypopharyngeal cancer with a 5-year minimum follow-up is presented. Occult metastasis was observed in 30% of the patients. Extracapsular spread was present in 39% of the positive nodes. The neck recurrence rate was 5.2%. Surgical specimens from cases of neck recurrence in pN0 necks were reevaluated for micrometastasis by immunostaining with antibody for cytokeratins. The immunohistochemical findings were positive in 1 of 4 cases. Functional neck dissection provides good neck control and survival rates for the cN0 neck. The accurate prognostic significance of extracapsular spread in cN0 necks is still unknown. Micrometastasis alone may be insufficient to explain recurrences in pN0 necks.

KEY WORDS — extracapsular spread, micrometastasis, N0 neck, neck dissection, neck recurrence.

INTRODUCTION

No agreement exists about the best management of the clinically negative (cN0) neck. Treatment strategies include watchful waiting and treating the neck when clinical metastasis develops; prophylactic irradiation; and elective neck dissection. Because a considerable number of cN0 necks have occult lymph node metastasis, expectant treatment of the neck may result in development of neck recurrence, which is not always easily curable.[1] This has led many specialists to offer elective treatment for patients with a cN0 neck. Deciding between prophylactic irradiation and elective neck dissection depends on several factors, but mainly on treatment of the primary tumor, the experience of the institution, and the patient's preference.

Some controversies concerning elective neck dissection need to be clarified. The so-called functional neck dissection (FND) consists of removal of lymphatic tissue of the neck while dissecting along fascial planes, thus sparing the spinal accessory nerve, the sternocleidomastoid muscle, and the internal jugular vein.[2] The number of nodal regions included in the resection does not have any bearing on the concept of a functional approach. The approach that we perform for laryngeal and hypopharyngeal cancer includes levels II to V. To be safe, FND requires all metastatic disease to be confined within the lymphatic system. Thus, it is a good approach in patients with cN0 necks and/or those with small, mobile nodes.

Neck recurrences usually appear in pathologically positive necks. However, in some instances neck recurrence develops in pathologically negative (pN0) necks. The presence of undetected micrometastases has been proposed by some as an explanation for this event.[3]

Extracapsular spread (ECS) is generally considered an ominous prognostic factor in palpable neck metastases.[4] However, the implications of ECS in small nodes, such as those that appear in cN0 necks, have not been sufficiently studied.

This retrospective clinical study evaluates the effectiveness of FND in providing regional control of metastatic disease in the cN0 neck in patients treated for laryngeal and hypopharyngeal cancer. Specifically, it addresses two unresolved issues related to the surgically treated N0 neck — neck recurrence in a pN0 neck and the significance of ECS in false-negative nodes — in terms of recurrence and survival rates.

PATIENTS AND METHODS

Between January 1984 and December 1994, 184 patients with laryngeal or hypopharyngeal squamous cell carcinoma were surgically treated at La Paz University Hospital in Madrid, Spain. All of them were

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TABLE 1. DISTRIBUTION OF PATIENTS ACCORDING TO PRIMARY SITE AND STAGE OF DISEASE

	Stage of Disease				
Primary Site	*T1*	*T2*	*T3*	*T4*	*Total*
Supraglottis	14	30	42	29	115
Glottis		5	14	1	20
Subglottis				3	3
Transglottis		4	12		16
Hypopharynx	3	4	9	2	18
Total	17	43	77	35	172

TABLE 3. NODAL METASTASES AND ECS ACCORDING TO STAGE OF PRIMARY TUMOR

	Nodes		*ECS*		
Stage	*Positive*	*Negative*	*Yes*	*No*	*NS*
I (T1N0)	4 (23%)	13 (76%)	1 (33%)	2 (66%)	1
II (T2N0)	9 (21%)	34 (79%)	1 (14%)	6 (86%)	2
III (T3N0)	25 (33%)	52 (67%)	11 (44%)	14 (56%)	
IV (T4N0)	13 (37%)	22 (63%)	5 (45%)	6 (55%)	2
Total	51	121	18	28	5

ECS — extracapsular spread, NS — not stated.

cN0 and underwent FND as a part of their treatment. Among these patients, 12 were lost to follow-up before completion of the 5-year period, thus leaving 172 patients with 253 dissected fields for the study. Clinical, pathological, and therapeutic information was reviewed from the medical records. The group included 170 men and 2 women with a median age of 60 years (range, 36 to 80 years). The primary tumor location included 156 laryngeal lesions and 18 hypopharyngeal (pyriform sinus) lesions. All patients were classified according to the 1987 UICC TNM classification (Table 1).

The surgical technique of FND has been previously described[5,6] and will not be detailed here. Ninety-one patients underwent ipsilateral FND, and bilateral FND was performed in 81 patients. Postoperative irradiation was indicated in 37 patients on the basis of pathological findings. Until 1990, postoperative radiotherapy was used in all cases with positive nodes. Since 1991, radiotherapy has only been used in patients with positive or close surgical margins. In the past decade, no postoperative radiotherapy was used in FND patients for nodal reasons. A total dose of 6,000 cGy was delivered on a schedule of 200 cGy/d, 5 d/wk.

Pathology reports were reviewed to identify the total number of lymph nodes, the number of positive nodes, and the presence or absence of ECS. In case of a neck recurrence in a previously pN0 neck, the surgical specimen was reexamined to search for micrometastasis. Additional histopathologic examination consisted of serial sectioning of paraffin-embedded lymph nodes. Each paraffin block was sectioned into 1-µm-thick sections. At least 6 slices from different levels were obtained for hematoxylin and eosin staining, and at least 3 slices for immunohistochemical analysis. Immunohistochemical analysis for cytokeratins was performed with the Dako EnVision+Plus System with peroxidase (diaminobenzidine) (Dako A/S, Glostrup, Denmark). A heat-induced antigen retrieval step was performed by incubating deparaffinized sections immersed in 0.01 mol/L citrate buffer (pH 6.0) in a pressure cooker for 3 minutes. The mouse monoclonal AE1/AE3 pan-cytokeratin antibody (Signet Laboratories, Inc, Dedham, Massachusetts) was applied at a 1:100 dilution. All patients were followed for a minimum of 5 years or until death. The site of recurrence was considered to be the most proximal site. The SPSS program (Statistical Package for the Social Sciences Plus) was used for statistical analysis. Survival and recurrence-free rates were determined with the Kaplan-Meier method, and the log-rank test was used for univariate testing of potential prognostic factors. A p value of less than .05 was considered to have statistical significance.

TABLE 2. SURVIVAL RATES ACCORDING TO PATHOLOGICAL NODAL STATUS

	n	*Median Survival (mo)*	*5-y Overall Survival Rate*	*5-y CSSR*
pN0	121	146	74%	86%
pN+	51	24	37%	53%

CSSR — cancer-specific survival rate.

RESULTS

The median overall survival time for the 172 patients was 144 months. After a 5-year minimum follow-up, 102 patients (59%) were alive, 97 of whom (56%) were without recurrence; 39 (23%) were dead of tumor (24 local, 7 neck, and 8 distant recurrences); and 31 (18%) had died of other causes. The relapse-free survival rate was 73%, and the cancer-specific survival rate was 76%. Table 2 shows the outcome of patients according to pathological N status. Nodal metastasis was significantly associated with a worse outcome.

Fifty-one patients had positive nodes. Therefore, the occult metastatic rate was 30%. The rate of ECS in the occult-positive lymph nodes was 39% (18 of 46). In 5 cases, the surgical specimen was not available for evaluation of this feature. Table 3 shows the incidence of metastatic cervical nodes and ECS according to the tumor stage. Stage III and IV patients had a higher incidence of positive nodes and ECS compared with stage I and II patients, although this result had no statistical significance. There were 9 regional recurrences (Table 4). Most recurrences (67%) arose in patients with advanced (T3 or T4)

TABLE 4. PATIENTS WITH NECK RECURRENCE

	Tumor	*FND*	*pN*	*N0 Nodes*	*ECS*	*Site*	*Recurrence*	*Time to Recurrence (mo)*
1	Pyriform sinus T2	Bilateral	+	4	+	NS	Dissected	5
2	Subglottic T4	Unilateral	–	–	–	PTR	Dissected	88
3	Subglottic T4	Unilateral	+	1	–	PTR	Dissected	11
4	Pyriform sinus T1	Bilateral	+	2	+	UJC	Dissected	11
5	Glottic T3	Unilateral	+	4	+	MJC	Dissected	12
6	Glottic T3	Unilateral	+	1	–	NS	Dissected	25
7	Subglottic T2	Unilateral	–	–	–	LJC	Dissected	39
8	Pyriform sinus T4	Unilateral	–	–	–	UJC	Undissected	39
9	Subglottic T4	Unilateral	–	–	–	MJC	Undissected	16

FND — functional neck dissection, NS — not stated, PTR — posterior triangle region, UJC — upper jugular chain, MJC — middle jugular chain, LJC — lower jugular chain.

primary tumors. Of the 9 patients in whom neck recurrence developed, only 3 were alive 5 years after surgery. One was treated with surgery alone, and 2 with surgery and radiotherapy. Four of the 9 neck recurrences (44%) appeared more than 2 years after the initial treatment. Seven recurrences were in treated fields, all of them within the operative field, and 2 developed in untreated fields, that is, on the opposite side of the neck.

Neck recurrences developed in 5.2% of the patients (9 of 172) and in 2.8% of the dissected fields (7 of 253). The remaining 2 recurrences developed on the contralateral, undissected part of the neck. Despite the negative histologic findings, neck recurrences developed in 4 patients with pN0 necks, 2 of them on the dissected side and 2 on the contralateral undissected side. Histologic examination of the hematoxylin-eosin sections showed no metastatic cells. Immunohistochemical study demonstrated the presence of micrometastasis in the surgical specimen of only 1 of these 4 patients (patient 8; Table 4). Focal positivity for pankeratins AE1-AE3 was demonstrated in isolated epithelial cells from a 6-mm-diameter node.

The total number of nodes and the number of positive nodes had no significant influence on prognosis. Of the 5 pathologically positive patients with neck recurrence, 3 had ECS. All patients in whom distant metastasis developed had ECS. In a comparison of cancer-specific survival rates between patients with and without ECS, those with ECS had poorer outcomes than patients with positive nodes without ECS (p = .003; Table 5). Radiotherapy had no significant influence on survival rates either in the whole group or in the ECS group.

TABLE 5. SURVIVAL RATE ACCORDING TO ECS

	No Radiotherapy			*Radiotherapy*		
	n	*Median Survival (mo)*	*5-y CSSR*	*n*	*Median Survival (mo)*	*5-y CSSR*
ECS	5	18	20%	13	17	25%
No ECS	14	72	75%	14	120	64%

DISCUSSION

Controversy exists regarding the treatment strategies for the cN0 neck. The use of careful observation, irradiation, or elective neck dissection depends mainly on personal and institutional preferences. The intention of this article was to assess the efficacy of FND in controlling metastatic disease to the cN0 neck, specifically focusing on two major controversies: neck recurrence in the pN0 neck and the role of ECS in the cN0 neck.

The neck nodal status is considered to be the most important single prognostic factor in the treatment of head and neck squamous cell carcinoma. The presence of a positive lymph node leads to a 50% reduction in the 5-year survival rate.[7] Our results (Table 1) support this statement. In fact, recurrences in the dissected neck are a serious event, and salvage is difficult. In our study, two thirds of the patients in whom neck recurrence developed were dead within 5 years. Therefore, neck treatment is mandatory when the risk of metastasis is high. Although imaging techniques have significantly improved, clinical examination continues to be the basis for therapeutic decision-making in cN0 patients. Much effort has been expended trying to correlate various features of the primary tumor (site, stage, differentiation) with the incidence of occult disease in the neck. However, the literature has not shown strict correlation of neck metastasis with any of the primary tumor factors studied to date.[8] The high occult metastatic rate in our study (30%) is in concordance with most series[9-12] and contributes to support elective neck treatment. Until imaging techniques provide an accurate diagnosis of all positive nodes, histopathologic examination will remain the most reliable method of assessing the precise metastatic status in patients with cN0 necks.

The neck recurrence rate in cN0 patients treated with elective dissection varies widely (4% to 20%), depending on the site of the primary tumor and the incidence of occult metastasis.[13-15] Interestingly, 4 of the 9 neck recurrences (44%) in this series developed more than 2 years after the initial treatment. It has been stated that most recurrences in the neck develop within 2 years.[9,16] Our results suggest that recurrences in patients with laryngeal and hypopharyngeal cancer can develop later. This fact emphasizes the importance of long-term follow-up in all cases.

Recurrence in the pN0 neck is one of the most striking events that can occur after neck dissection. In our series, recurrences developed in 4 patients (3.3%): 2 in dissected fields and 2 in undissected fields. The incidence of recurrence in dissected pN0 necks varies from 4% to 29%.[10,12,14,15] This feature is sometimes not stated in neck dissection studies, and a definitive explanation has not yet been proposed for such a curious event. The presence of micrometastasis not found on routine histopathologic examination has previously been described in head and neck tumors.[3] Detection of micrometastasis by a pathologist is enhanced by a large number of sections per lymph node and can be done by immunohistochemical analysis.[17,18] An article by Ferlito et al[19] supports the evidence that micrometastases have clinical and prognostic implications. Weissler et al[12] found a 6% neck recurrence rate in pN0 necks and suggested the presence of micrometastasis as the explanation for the failure rate in the pN0 dissected necks. However, as FND has proved to be therapeutically effective for N0, N1, and some N2 necks,[20] it appears reasonable that it should also be able to control pN0 necks with micrometastasis. To verify this hypothesis, we reexamined the surgical specimens of the 4 cases of recurrence in pN0 necks. Micrometastasis was detected in only 1 case. Although no definitive conclusion can be extracted from this finding, micrometastasis may not be enough to explain all cases of recurrence in the pN0 neck. This interesting finding could be related to reseeding of the neck from an unrecognized recurrent tumor or second primary, or from nondissected retropharyngeal nodes.[21] Recurrence in the context of a pN0 neck continues to be an unresolved issue. Recurrences in the undissected neck are sometimes not reported or are not considered as recurrences.[10,12,14] They probably reflect incorrect planning of the extent of surgery.

The prognostic significance of ECS in patients with positive neck nodes is well known.[4,7,22] However, the implications of ECS in nonpalpable nodes are not so well documented. It has been stated that the risk of recurrence is not significantly higher when the disease invades the nodal capsule or breaks it microscopically than when there is no ECS at all.[23,24] Therefore, massive metastatic lesions that have broken the capsule of the cervical node should be differentiated, in terms of recurrence and prognosis, from small nodes with microscopic rupture of the capsule. Alvi and Johnson[14] found that ECS in occult metastasis was an indicator of poor prognosis in patients with N0 necks treated with neck dissection. They found ECS in 49% of patients with occult metastasis. Neck recurrence and distant recurrence were not significantly associated with ECS. Pitman et al,[10] in a similar study, found a higher incidence of distant metastasis and a trend toward decreased survival rate in cN0 patients with ECS (33% rate of ECS in occult positive lymph nodes) as compared with those with positive nodes without ECS. Neck recurrence was not significantly associated with the presence of ECS. In our study, ECS was found in 35% of falsely cN0 necks. These patients had a lower survival rate than those without this feature, despite not having a significantly higher local, regional, or distant recurrence rate. These results suggest that ECS may have some prognostic significance in patients with cN0 necks (ie, with small occult metastatic nodes), but its value as a predictor of neck recurrence may not be as obvious as in patients with palpable nodes with macroscopic ECS. Whether this feature represents increased tumor aggressiveness or depressed host immunocompetence is still unknown.

CONCLUSIONS

The high incidence of occult metastasis justifies the performance of a surgical procedure such as FND, because pathological examination is the only definitive way of determining actual involvement of cervical lymph nodes by cancer. Functional neck dissection is a reliable, nonaggressive surgical technique for the N0 neck that provides acceptable neck control and survival rates. Recurrence in the pN0 neck may not be explained only by the presence of micrometastases, but may also involve some unknown factors, probably related to host immune surveillance. The presence of ECS in a false-negative node appears to have prognostic value, although its precise predictive value is still unknown.

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COMPLICATIONS OF INTRANASAL PRESCRIPTION NARCOTIC ABUSE

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The abuse of drugs via an intranasal route is an increasingly prevalent pattern of behavior. In the past year, a number of patients received care at our institution for complications resulting from the previously unreported phenomenon of intranasal prescription narcotic abuse. This report describes the clinical manifestations of this form of drug abuse in 5 patients. Their symptoms consisted of nasal and/or facial pain, nasal obstruction, and chronic foul-smelling drainage. Common physical findings were nasal septal perforation; erosion of the lateral nasal walls, nasopharynx, and soft palate; and mucopurulent exudate on affected nasal surfaces. In addition, 2 of the 5 patients had invasive fungal rhinosinusitis, which appears to be a complication unique to intranasal narcotic abuse.

KEY WORDS — drug abuse, codeine, fungus, hydrocodone, nose, oxycodone, sinusitis.

INTRODUCTION

Intranasal drug abuse has been increasing in recent years, with cocaine as the most prevalent drug used by this route. This form of abuse results in nasal congestion and inflammation, epistaxis, and erosion of the nasal septum and other structures.[1] Intranasal abuse of other drugs, such as heroin and methamphetamine, has been described to a lesser extent, with symptoms and complications similar to those of intranasal cocaine use.[2] We recently encountered 5 patients who abused prescription narcotics in a similar fashion, which has previously not been described in the medical literature. In this report, we describe the representative clinical course of 1 of these patients, and summarize the presentation and sequelae of intranasal prescription narcotic abuse according to our experience.

CASE HISTORY

A 26-year-old woman (case 4) had a 4-month history of progressive nasal pain, epistaxis, odynophagia, dysphagia, and weight loss. She also reported a persistent musty odor and foul-smelling discharge from her nose. These symptoms persisted despite multiple courses of oral antibiotics. Although she initially denied any history of drug abuse, she later admitted that she regularly snorted crushed hydrocodone bitartrate–acetaminophen tablets, and that she had used cocaine in the past. When asked why she abused hydrocodone-acetaminophen in this fashion, she stated that she experienced a more rapid and intense euphoria than that produced by taking the pills orally. Human immunodeficiency virus, sweat chloride, and immunoglobulin test results were negative.

Physical examination showed that she had hypernasal speech and right nasal and maxillary pain upon palpation. The mucosa of the nasal septum and the right lateral nasal wall was covered with a purulent exudate and appeared necrotic. A large nasal septal perforation was also present. The soft palate and uvula were partially eroded and covered with mucopurulent debris. A sinus computed tomography scan demonstrated a large septal perforation, mucosal thickening within the right maxillary sinus, and thickening of the lateral nasal wall (Fig 1). She underwent surgical nasal and sinus endoscopy with extensive debridement of the necrotic nasal mucosa. Histopathologic evaluation by Gomori's methenamine silver staining showed numerous fungal organisms with sep-

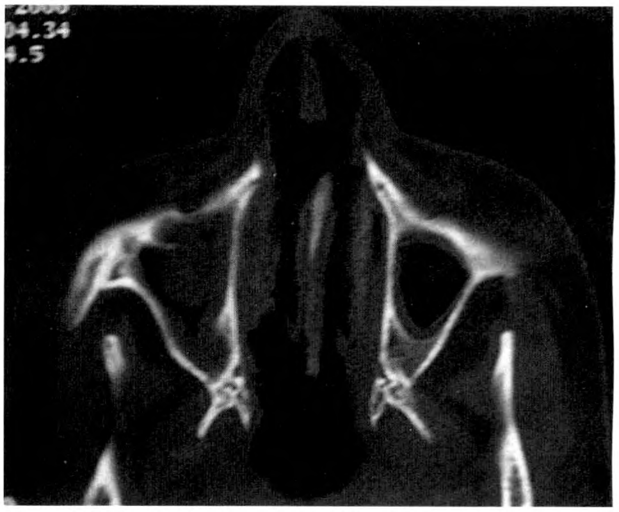

Fig 1. (Case 4) Axial sinus computed tomography scan shows nasal septal perforation and lateral nasal wall abnormalities due to invasive fungal rhinosinusitis.

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CLINICAL FEATURES OF PATIENTS WITH COMPLICATIONS OF INTRANASAL PRESCRIPTION NARCOTIC ABUSE

Case	*Age (y)*	*Sex*	*Intranasal Drug Use*	*Drug Testing*	*Symptoms*	*Physical Findings*	*Surgical History*	*Fungal Cultures*	*Fungal Disease*
1	24	M	Hydrocodone bitartrate, codeine phosphate, nonprescription cold remedies	None documented	Nasal obstruction, foul-smelling nasal crusting and discharge, velopharyngeal insufficiency, obstructive sleep apnea	Septal perforation, erosion of inferior, turbinates, soft palate erosion, mucopurulent exudate	Nasal and sinus endoscopy and debridement, septectomy	None documented	None documented
2	37	F	Cocaine phosphate, oxycodone hydrochloride, hydrocodone bitartrate, methadone hydrochloride	Urine drug screen for hydrocodone, oxycodone, methadone, benzodiazepine metabolites; nasal crusting drug screen for methadone, hydrocodone, oxycodone	Nasal obstruction, purulent nasal drainage	Septal perforation, mucopurulent crusting on septum and turbinates, nasopharyngeal ulcer	Nasal and sinus endoscopy and debridement	*Candida krusei*; *Torulopsis glabrata; Aspergillus fumigatus*	Noninvasive
3	32	F	Cocaine phosphate, hydrocodone bitartrate	None documented	Nasal congestion, nasal pain, headaches, weight loss	Saddle nose deformity, septal perforation, mucopurulent exudate	Nasal and sinus endoscopy and debridement	*Penicillium*; *Aspergillus* species	Noninvasive
4	26	F	Hydrocodone bitartrate	None documented	Nasal pain, epistaxis, odynophagia, dysphagia, hypernasal speech	Septal perforation, soft palate erosion, mucopurulent debris on septum and lateral nasal walls	Nasal and sinus endoscopy and debridement	*Aspergillus flavus*; *Veillonella* species	Invasive rhinosinusitis
5	32	F	Hydrocodone bitartrate, codeine phosphate, oxycodone hydrochloride	None documented	Nasal and facial pain, purulent nasal drainage	Septal perforation, purulent exudate on lateral nasal walls and nasopharynx	Nasal and sinus endoscopy and debridement	*Aspergillus* species	Invasive rhinosinusitis

tate hyphae within the nasal mucosa, confirming a diagnosis of invasive fungal rhinosinusitis. Tissue cultures from the nose and nasopharynx eventually grew *Aspergillus flavus* and *Veillonella* species. After the procedure, she remained in the hospital for treatment with intravenous amphotericin B. The patient underwent endoscopic nasal debridement 3 more times over the course of the following 5 weeks while treatment with amphotericin B continued. The histopathology from these subsequent procedures continued to show septate fungal hyphae within debris, although there was no longer any evidence of invasion. After the patient was discharged, she did not keep her follow-up appointments and was lost to follow-up.

DISCUSSION

The clinical findings of all 5 patients encountered are summarized in the Table. Common patient complaints included nasal obstruction, irritation, crusting, nasal and/or facial pain, and foul-smelling purulent nasal discharge. Velopharyngeal insufficiency was also seen when erosion of the soft palate was present. These patients suffered from both acute and chronic damage to the nasal passageways, which extended to the nasopharynx and oropharynx in some of the more severely affected individuals. Among the acute findings, inflammatory changes of the mucosa were common and were often accompanied by ulceration and necrosis. A thick, foul-smelling crust was usually present on affected mucosa. In case 3, recently insufflated crushed pill material was seen to be adherent to the nasal mucosa (Fig 2). Chronic changes consisted of erosion of the inferior turbinates, uvula, and soft palate, as well as perforation of the nasal sep-

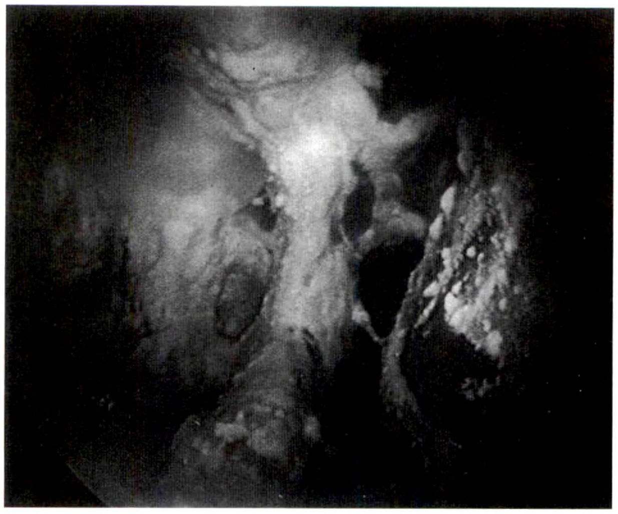

Fig 2. (Case 3) Nasal endoscopy shows active intranasal narcotic abuse; ie, white powdery residue from crushed pills is intermixed with crusted debris covering septal perforation and turbinate mucosa.

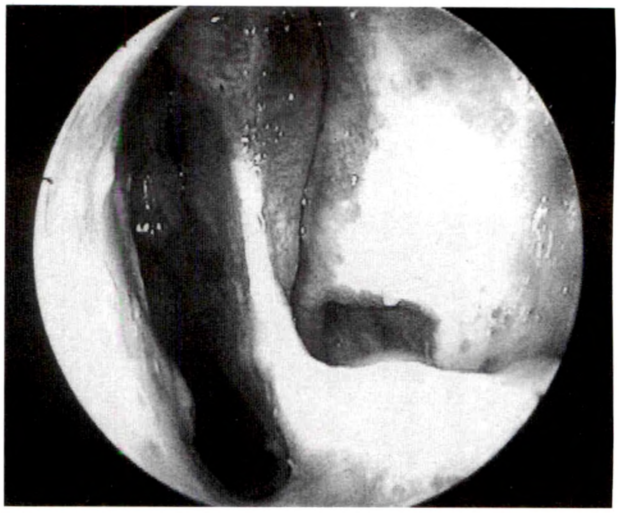

Fig 3. (Case 1) Nasal endoscopy shows large central perforation and scarred mucosa along inferior turbinates and septum.

tum (Fig 3). These findings are similar to those commonly associated with intranasal cocaine abuse.[3,4] The symptoms of these patients could have easily been attributed to cocaine abuse, especially since 3 patients admitted a previous history of the latter. However, all patients admitted to active intranasal abuse of prescription narcotics.

In addition to the acute and chronic inflammatory changes described above, 2 of our patients (cases 4 and 5) also had invasive fungal *Aspergillus* rhinosinusitis. Although 2 other patients (cases 2 and 3) had fungal elements within the intranasal exudate and debris, fungal invasion, fortunately, did not occur. Fungal sinusitis is normally caused by opportunistic pathogens such as Phycomycetes (*Mucor*, *Rhizopus*) and *Aspergillus* species. Among the numerous *Aspergillus* species, *Aspergillus fumigatus* is the most common infective agent, followed by *Aspergillus niger* and *A flavus*. *Aspergillus* species are widespread throughout the environment and can become pathogenic after inhalation. As a result, the lungs and paranasal sinuses are the most commonly infected areas. The clinical manifestations of fungal rhinosinusitis include bloody nasal discharge and black or necrotic nasal mucosa. Definitive diagnosis of fungal infection is made by histologic identification using Gomori's methenamine silver staining on biopsy specimens.[5]

Invasive or lethal fungal infections are uncommon and normally occur only among immunocompromised patients.[6] None of our patients suffered from any demonstrable type of chronic disease, and there was no objective evidence to indicate an immunocompromised state. Invasive fungal rhinosinusitis has not been reported as a complication of intranasal cocaine use, so it appears as though this is a complication unique to intranasal prescription narcotic abuse. Contrary to the aggressive nature of invasive fungal rhinosinusitis seen in immunocompromised patients, the progression of disease in our patients was fairly indolent, with symptoms slowly worsening over a matter of months instead of days. The only symptom that seemed to differentiate the patients with invasive versus noninvasive fungal rhinosinusitis appeared to be the severity and progressive nature of nasofacial pain in the patients with invasive disease. These 2 patients responded well to nasal and sinus debridement and intravenous antifungal therapy and had no orbital or intracranial complications. As for the 2 patients who had fungal organisms present without evidence of invasive disease, it is unclear whether the cultured fungi were actual pathogens contributing to the patients' overall symptoms, or whether they were merely saprophytic organisms.

At this point, we do not know how individuals with apparently normal immune systems could develop invasive fungal rhinosinusitis. One possible explanation for this complication is that the nasally inhaled narcotics cause some sort of localized immunosuppression.[7] Morphine use and its potential association with increased infections has been the subject of several prior studies. Lymphocytes and macrophages both possess opiate receptors, and the effects of morphine upon the human immune system may be regulated by an opioid receptor–dependent mechanism.[8] In light of the potential immunosuppressive effects of morphine, we must consider the possibility that the intranasal abuse of prescription narcotics may cause localized immunosuppression that supports the growth of fungal organisms. There may also have been additional influences, such as nutrition and lifestyle factors promoting the development of fungal disease.

In retrospect, a number of issues related to the care of the drug-dependent patient arose. Only 1 individual (case 2) underwent thorough urine drug screening before treatment. Complete drug screening identifies the actual drugs a patient is actively abusing at the time of treatment, and can alert a physician toward possible problems with withdrawal.[9] In addition, close observation of these patients within a secure environment should be considered during their hospital stay to prevent them from obtaining drugs from their friends or suppliers. A disturbing trend among the described patients is that only 1 of the 5 patients returned for follow-up care in our clinic. Their tendency to continue narcotic abuse and their apparent disregard for their well-being places this patient population at risk for continued problems and complications. A consultation with a drug counselor or a specialist in addiction medicine might have helped these individuals with postoperative compliance, in addition to prompting the initiation of long-term therapy for their drug dependence.[10,11]

CONCLUSION

These 5 cases represent the previously unreported phenomenon of intranasal prescription narcotic abuse. This practice results in acute inflammation that results in nasal or facial pain, crusting, and epistaxis. The chronic changes range from septal perforation to turbinate and soft palate erosion. A more serious complication is the development of fungal rhinosinusitis. Four of the 5 patients described had fungal organisms identified upon culture; 2 patients developed invasive fungal disease, a condition normally seen only in immunocompromised patients and not previously described in association with the chronic intranasal abuse of cocaine or other drugs. Fortunately, both patients responded well to surgical debridement and intravenous antifungal therapy.

Because intranasal prescription narcotic abuse was previously an uncharacterized entity, no consistent form of management was undertaken for the described patients. In retrospect, useful recommendations that can be drawn from these representative cases are as follows.

1. Intranasal prescription narcotic abuse should be considered as a possible cause of intranasal tissue erosion and crusting, even in patients with a history of previous cocaine abuse.

2. A complete urine drug screen should be performed as part of the initial evaluation of patients in whom intranasal narcotic abuse is suspected. Drug screening may also be performed on the exudate present within the nasal passageways.

3. Consultation with an addiction medicine specialist would be valuable in the acute management of drug abuse and possible complications from withdrawal; in addition, this could also aid patients to comply with postoperative care and to seek therapy for their drug dependence.

4. Given the considerable probability of abuse of multiple drugs by these patients, which may include intravenous drug use, screening for human immunodeficiency virus and hepatitis should be performed.

5. During surgical debridement, fungal cultures and stains should be performed, in addition to routine cultures and biopsies of affected nasal areas, to determine the presence or absence of fungal rhinosinusitis.

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GASTROESOPHAGOPHARYNGEAL REFLUX IN PATIENTS WITH CONTACT GRANULOMA: A PROSPECTIVE CONTROLLED STUDY

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The aim of this study was to compare the incidences of gastroesophagopharyngeal reflux in patients with contact granuloma and healthy controls. A 24-hour ambulatory esophagopharyngeal pH monitoring technique was used to measure reflux parameters in the pharynx and distal esophagus. Pharyngeal acid reflux events occurred in 17 of 26 granuloma patients (1 to 20 episodes per patient) and 5 of 19 controls (1 to 8 episodes per patient). The reflux episodes were typically short and occurred predominantly in an upright position. A comparison between the groups showed a significant difference in the number of pharyngeal reflux episodes ($p = .009$) and in the total time of pH below 4 ($p = .006$). On the other hand, we found no significant differences in any esophageal reflux parameters, except for the percentage of distal esophageal reflux episodes that reached the pharynx ($p = .006$). In this study, pharyngeal acid exposure was significantly more prevalent in patients with contact granuloma than in healthy controls.

KEY WORDS — contact granuloma, gastroesophagopharyngeal reflux, pH monitoring.

INTRODUCTION

Contact granuloma is a relatively rare granulomatous inflammation of the posterior glottis that occurs mostly in men. The most common symptom is hoarseness combined with persistent throat clearing, sore throat, and globus sensation. Despite its infrequent occurrence, contact granuloma has received considerable attention in the literature because of frustration encountered in its clinical management. The healing time is long, regardless of treatment, and the recurrence rate after surgery is greater than 90%.[1] It is not known whether contact ulcer and granuloma are separate entities or different stages of the same pathological process, but the terms are often used interchangeably.

The association between laryngeal disease and gastroesophageal reflux (GER) was first suggested in 1968, by Cherry and Margulies,[2] who reported on 3 patients with contact ulcer of the larynx and significant esophageal reflux on barium swallow studies. They suggested that laryngeal overspill of acid was a supplementary factor to vocal abuse in contact ulcer formation. Delahunty and Cherry[3] supported this theory by the experimental induction of laryngeal granulomas.

The first series of patients with contact granulomas evaluated for GER by modern diagnostic methods, such as barium esophagography, esophageal manometry, and pH monitoring, was reported in 1983 by Öhman et al.[4] In their study, 74% of patients had abnormal reflux. Furthermore, Svensson et al[5] found that 9 of their 13 patients with idiopathic granuloma had esophagitis, GER, or motility disturbances. Goldberg et al[6] and Ward et al[7] were the first to document the benefit of antireflux therapy in contact ulcer patients. Nowadays, GER is thought to be the cause of a variety of otolaryngological symptoms and disorders. Reflux into the pharynx has been previously documented in patients with chronic hoarseness,[8,9] posterior laryngitis,[10-12] vocal fold nodules,[13] subglottic stenosis,[10] and laryngeal cancer.[10] However, the frequency and pattern of pharyngeal acid reflux in contact granuloma patients have not been systematically studied, and only a few studies have included pH monitoring in the pharynx in healthy controls.[10,12,13]

Ambulatory 24-hour pH monitoring is currently the best single method for diagnosis of gastroesophageal reflux disease (GERD).[14-16] Combined esophageal and hypopharyngeal pH monitoring offers the opportunity to document not only GER, but also gastroesophagopharyngeal reflux. Some studies have shown that many patients in otolaryngological practice have a reflux pattern that is distinctly different from the pattern seen in patients with reflux esophagitis.[10] Pharyngeal reflux is mainly occult in these patients and may therefore lead to a long delay in the diagnosis and treatment of the underlying disease.

The aims of the present study were to determine and compare the incidences of gastroesophagopharyngeal reflux in patients with contact granuloma and

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in normal controls.

MATERIAL AND METHODS

Subjects. From February 1997 to February 1999, 28 consecutive patients with contact granuloma were studied. Patients with contact ulcer or those with granuloma of known cause, such as intubation, were excluded. Technically adequate registrations could be obtained in 26 of the 28 patients. These 26 patients (24 men and 2 women), 37 to 74 years of age (mean, 51.8 years), all nonsmokers, were included in the study. The granuloma patients reported the presence of the following symptoms: frequent clearing of the throat (50%), hoarseness (54%), sore throat (81%), and heartburn and/or regurgitation (48%).

During the study period, we also evaluated 20 vocally healthy volunteers recruited by advertisement. One of them was excluded from the later data analysis, because the patient was unable to tolerate ambulatory pH monitoring for a full 24 hours. The remaining 19 healthy volunteers (16 men and 3 women) were 20 to 51 years of age (mean, 31.8 years). None of the healthy volunteers were taking drugs known to alter esophageal motor function or gastric acid secretion. They had no history of heartburn, acid regurgitation, or epigastric pain. No upper gastrointestinal endoscopy or barium swallow studies were performed before the study. No controls had a history of alcohol abuse, diabetes mellitus, or vascular or neurologic disease. Only 1 of them smoked. At laryngoscopic examination, all controls had normal laryngeal mucosa or changes that were not considered pathological.

The study was approved by the Ethical Committee for Human Research at Huddinge University Hospital.

Laryngoscopic Examination. The patients underwent a video laryngoscopic examination between 3 and 6 weeks before the manometry and pH monitoring, whereas the healthy controls underwent all the examinations on the same day. The subjects were examined with a rigid telescope (Storz 8706 CJ, 70°) or with a flexible fiberscope (Olympus ENF-P3, 3.5 mm). The endoscopes were connected to a light source (Brüel & Kjær 4914 or Wolf 5052) and to video equipment (Panasonic G1E camera; Panasonic AG-7350 Super-VHS video recorder; Sony CVM-1810E monitor). The subjects were anesthetized in the nose with naphazoline hydrochloride–lidocaine hydrochloride (0.17 mg/mL and 3.4 mg/mL) as needed. To confirm the diagnosis, all of the recordings were later examined by another experienced phoniatrician.

Manometry. The granuloma patients and the healthy controls initially underwent esophageal manometry. The locations of the upper esophageal sphincter (UES) and the lower esophageal sphincter (LES) were determined, and the resting pressure of the LES (millimeters of mercury), as well as esophageal peristaltic activity, was evaluated. We used a low-compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialties Inc, Greendale, Wisconsin) and an 8-lumen polyvinyl catheter (Synectics Medical Inc, Stockholm, Sweden; diameter, 4.5 mm), with the 3 distal orifices placed at the same level and the others placed 5 cm apart. The catheter was perfused with distilled water at a rate of 0.5 mL/min, and pressures were registered with Gould P50-150 transducers. Data were collected and analyzed with Polygram v 4.60 C2 (Synectics Medical Inc). The manometry catheter was inserted transnasally into the stomach with the patient in a sitting position, and the manometry was performed with the patient in a supine position. A stationary pull-through technique was used to record the LES resting pressure in the 3 distal orifices. The catheter was withdrawn in 1-cm increments into the esophagus. The data for each patient represent the mean of 2 individual pull-throughs, measured from end-expiration gastric baseline to the highest end-expiration esophageal sphincter pressure. The normal resting pressure for the LES at our laboratory is 10 to 30 mm Hg. As the catheter was withdrawn, esophageal peristalsis was evaluated on the basis of the mean values of 10 wet swallows (5 mL) given at 30-second intervals. The UES pressure was monitored during 3 dry swallows. Thus, we determined the distance between the LES and the UES and could choose a pH probe suitable for each patient. The esophageal manometry was performed after at least 4 hours of fasting.

Ambulatory 24-Hour pH Monitoring. After manometry, the subjects underwent ambulatory 24-hour dual-probe pH monitoring with 2 monocrystalline antimony pH probes positioned along a single catheter (Synectics Medical Inc; diameter, 2.1 mm). The 2 recording sites were spaced individually and located 15 to 24 cm apart. A silver–silver chloride cutaneous reference electrode was attached to the skin on the anterior part of the chest. Data were collected with a portable data logger (Digitrapper Mark III, Synectics Medical Inc) with a sampling rate of 4 seconds. Both probes were calibrated simultaneously in buffer solutions of pH 7 and pH 1 before each test. We placed the pH probes at 2 manometrically determined sites: the proximal sensor was positioned 1 cm proximal to the oral end of the UES, and the distal sensor, 5 cm above the oral end of the LES. The probes were secured with tape to the nose. The subjects were encouraged to eat their regular meals with restrictions for intake of drink or food with a pH of less than 4. They were also asked to abstain from smoking and drinking alcohol. All subjects kept a detailed diary indicating the time of oral intake, the changing of position (upright and supine), and symptomatic events such as heartburn or regurgitation. H2-receptor antagonists and proton-pump inhibitors were discontinued for a minimum of 10 days before the study. After the 24-hour monitoring period, the probe was removed and the data were transferred to a computer for the analysis with EsopHogram 5.70C2 (Synectics Medical Inc). In addition, pH recordings were displayed on-screen for a detailed manual analysis and determination of the temporal relationship of the pH declines registered at the 2 probe sites.

Definition of Reflux Episodes. For both sites, a decrease in pH to less than 4 that was not induced by eating or drinking was considered the beginning of a reflux episode, and the following rise to a pH of greater than 5 was considered the end of such an episode. For the pharyngeal probe, the decrease had to be abrupt and simultaneous with or preceded by a decrease in pH of similar or larger magnitude in the esophageal probe to be accepted as a pharyngeal reflux event. Determination of the temporal relationship between the onset of pH declines at both sites was performed. This differentiated pH declines induced by oral intake, aerodigestive tract residue and secretions, pharyngeal probe movement, or loss of mucosal contact in which the pharyngeal pH decline preceded esophageal pH drops from true gastroesophagopharyngeal reflux episodes. The information recorded on the data logger was compared with the information provided in the diary at the completion of the study. Calculation and evaluation of all recordings were done by one of the authors (R.Y.).

The results of the ambulatory pH study regarding reflux to the distal esophagus were considered abnormal if the pH variables exceeded 2 SD from the mean derived from the asymptomatic normal subjects studied in our laboratory. These values for esophageal monitoring were 7% upright, 3% supine, and 6.5% total.

Using esophageal manometry and 24-hour pH monitoring, we determined 1) the LES resting tone and the sphincter's responses to swallowing and the presence of esophageal dysmotility, 2) the number of pH declines to less than 4 in the distal esophagus and pharynx, 3) the percentage of esophageal reflux episodes that reached the pharynx, and 4) the acid exposure and average acid clearing times at the 2 recording sites. The total acid exposure time was defined as the time during which the distal esophagus or pharynx was exposed to acid. The percent acid exposure time was calculated as the percentage of total time during which the pH probe was exposed to a pH of less than 4. The average acid clearance time was derived by dividing the total acid exposure time in minutes by the number of reflux episodes at that site. The data from the proximal and distal pH sensors taken during supine and upright periods were analyzed independently. Statistical comparison of the LES resting pressures between the groups was performed with the *t*-test, because the data were normally distributed. All other comparisons were performed with the nonparametric Mann-Whitney test because of skewed distributions of pH data.

RESULTS

Normative Data. Pharyngeal acid exposure events occurred in 5 of the 19 healthy controls: 3 subjects had 1 episode each, 1 subject had 2 episodes, and 1 had 8 episodes during the 24-hour monitoring. All except 2 of these reflux episodes were registered in an upright position.

Manometric Findings. All healthy volunteers and 21 of the 26 patients with granuloma had normal esophageal peristalsis. Four granuloma patients with GERD had low-amplitude peristaltic pressure waves, and 1 had repetitive peristaltic contractions. The average LES resting pressure was 19.7 mm Hg (SD, 6.9 mm Hg) for the granuloma patients and 16.6 mm Hg (SD, 4.4 mm Hg) for the healthy controls. Both the granuloma patients and the healthy controls showed normal LES and UES relaxation in response to swallowing.

pH Monitoring in Distal Esophagus. Acid episodes in the distal esophagus were identified in all subjects, indicating adequate registration. Half of the granuloma patients (13/26) had normal esophageal pH monitoring results, and the other half had pathological pH results. Of the 19 healthy volunteers, 3 had esophageal pH recordings indicating increased acid reflux. The number of reflux episodes, total acid exposure time, percent acid exposure time, and acid clearance time showed considerable intersubject variability in each group. A comparison between granuloma patients and controls showed no significant differences in any esophageal reflux parameters, except in the percentage of distal reflux episodes that reached the pharynx (p = .006). Table 1 summarizes the pH data from the distal esophagus.

pH Monitoring in Pharynx. Pharyngeal acid reflux occurred in 17 of the 26 patients with granuloma. The number of pharyngeal reflux episodes in this group ranged from 1 to 20. However, of the healthy volunteers, 3 had 1 episode, 1 had 2 episodes, and another had 8 episodes of pharyngeal reflux. Of all of the pharyngeal reflux episodes, 93% occurred in the

TABLE 1. RESULTS OF ESOPHAGEAL 24-HOUR pH MONITORING OF GRANULOMA PATIENTS AND HEALTHY CONTROLS

pH Variables in Esophagus	*Granuloma Patients*	*Healthy Controls*	*p*
Total No. of episodes	48 (13-114)	38 (5-91)	.168
Upright	44 (13-103)	34 (5-87)	.260
Supine	3 (0-37)	3 (0-16)	.267
Episodes >5 min	2 (0-14)	1 (0-10)	.066
Longest episode (min)	14.0 (1-83.4)	5.3 (0.1-158.1)	.093
Total time pH < 4 (min)	68.5 (5.3-292.9)	30.6 (0.5-388.1)	.091
Upright	54.5 (4.2-237.1)	25.0 (0.5-137.9)	.129
Supine	2.3 (0-140.9)	1.9 (0-308.1)	.312
% Time pH < 4 total	5.0 (0.4-21.4)	2.2 (0.04-28.9)	.079
Upright	5.6 (0.6-27.2)	3.1 (0.06-17.4)	.198
Supine	0.4 (0-34.9)	0.4 (0-37.6)	.269
Total acid clearance time (min)	1.4 (0.3-3.7)	0.9 (0.1-6.0)	.081
Upright	1.1 (0.3-2.7)	0.8 (0.1-2.7)	.115
Supine	0.7 (0-21.9)	0.6 (0-19.3)	.396
% Episodes that reached pharynx	6.2 (0-28.5)	0 (0-8.8)	.006
LES resting pressure (mm Hg; mean ± SD)	19.7 ± 6.9	16.6 ± 4.4	.192

Except for lower esophageal sphincter (LES) resting pressure, data are medians; 5th and 95th percentiles are given in parentheses. "Episodes" — events in which pH < 4.

TABLE 2. RESULTS OF PHARYNGEAL 24-HOUR pH MONITORING OF GRANULOMA PATIENTS AND HEALTHY CONTROLS

pH Variables in Pharynx	*Granuloma Patients*	*Healthy Controls*	*p*
Total No. of episodes	2 (0-18)	0 (0-8)	.009
Upright	2 (0-20)	0 (0-6)	.006
Supine	0 (0-4)	0 (0-2)	.750
Longest episode (min)	0.1 (0-3.3)	0 (0-0.7)	.009
Total time pH < 4 (min)	0.2 (0-9.6)	0.0 (0-1)	.006
% Time pH < 4 total	0.02 (0-0.9)	0.0 (0-0.1)	.008
Acid clearance time (min)	0.1 (0.1-2.6)	0.0 (0-0.7)	.017

Data are medians; 5th and 95th percentiles are given in parentheses.

upright position, and 3 subjects, 2 from the granuloma group and 1 healthy control, had pharyngeal reflux episodes in both the upright and supine positions. The parameters of pharyngeal acid reflux are shown in Table 2. Comparison between the groups showed that pharyngeal reflux episodes occurred significantly more often in the granuloma patients than in the control group (p = .09). The durations of the reflux episodes in the 5 healthy subjects ranged between 6 and 12 seconds (mean, 9.0 seconds). The durations of the acid episodes in the granuloma group varied from 6 seconds to 3 minutes (mean, 28.8 seconds). In both groups, isolated pharyngeal pH declines were observed; also, anterograde pH declines from the pharynx to the distal esophagus were observed during oral intake. These episodes were disregarded as reflux episodes. Of a total of 279 acid episodes in the pharynx, 96 (34.4%) were accepted as reflux episodes, and the rest, 183 episodes (65.6%), were considered artifactual.

Based on our pH monitoring, 4 different subgroups of granuloma patients could be identified: 1) patients with normal 24-hour pH monitoring results (n = 4); 2) patients with normal distal esophageal monitoring results but reflux episodes to the pharynx (n = 9); 3) patients with abnormal distal esophageal monitoring results but no reflux episodes to the pharynx (n = 5); and 4) patients with abnormal monitoring results in the distal esophagus and reflux episodes to the pharynx (n = 8; Table 3). The 2 female patients with granuloma had normal pH monitoring results in the distal esophagus, as well as in the pharynx.

DISCUSSION

The data in this study confirm that pharyngeal reflux is more common in granuloma patients than in asymptomatic healthy subjects. The reflux episodes are typically short and occur predominantly in an upright position. This kind of reflux pattern has previously been found in patients with subglottic stenosis and laryngeal cancer,[10] vocal nodules,[13] and reflux laryngitis.[10,12] The short duration of the reflux episodes may explain the low incidence of classic reflux symptoms in patients with contact granuloma.

Early studies emphasized the role of mechanical trauma as an etiologic factor in granuloma formation.[17] This trauma was especially marked at low speech fundamental frequencies and during coughing and clearing of the throat. Thus, voice therapy

TABLE 3. BREAKDOWN OF pH STUDY RESULTS OF GRANULOMA PATIENTS VERSUS HEALTHY CONTROLS

pH Monitoring	*No. of Granuloma Patients (n = 26)*	*No. of Healthy Controls (n = 19)*
Normal distal esophageal pH without reflux into pharynx	4	14
Normal distal esophageal pH with reflux into pharynx	9	2
Pathological distal esophageal pH without reflux into pharynx	5	0
Pathological distal esophageal pH with reflux into pharynx	8	3

has traditionally been used as a treatment for contact granulomas. This treatment decreases hyperfunction, vocal fry, and monotony, and increases fundamental frequency in some patients,[18] but it has not shortened the healing time substantially.[1] Gastroesophagopharyngeal reflux may be a contributory factor in the persistence of granuloma, even though the cause-and-effect relationship between reflux and granuloma has not been established in this study. Controlled studies on antireflux treatment in granuloma patients are to be performed.

We did not find any significant difference between the groups with manometry. The LES resting pressures were almost similar in both investigated groups, and most of the subjects had normal esophageal peristalsis. Thus, esophageal manometry does not seem to be useful in the diagnosis of extraesophageal reflux. However, the pressure of the UES or the transient relaxations of the UES, which could not be evaluated by our method, may give us more information in the future about the pathophysiology of pharyngeal reflux.

Another predominant finding in this study was that a higher percentage of esophageal acid episodes reached the pharynx in the granuloma group than in the control group. There was no statistically significant difference in the number of distal reflux episodes in an upright position between these groups, and the majority of pharyngeal acid episodes occurred only in an upright position. Also of particular interest are the 9 patients who had normal esophageal monitoring results but had pharyngeal reflux associated with the physiological esophageal reflux episodes. This finding suggests that granuloma patients may have abnormalities in the mechanism that normally prevents gastroesophagopharyngeal reflux. Ulualp et al[19] have shown that stimulation of the human pharyngeal mucosa by irrigation of minute amounts of water stimulates the pharyngo-UES contractile reflex. They suggest that this reflex may be activated during pharyngeal reflux of gastric acid, thus increasing the UES pressure. It could be considered as one of the protective mechanisms against contact of acid refluxate with the hypopharyngeal and laryngeal structures. In the same study, they showed that the volume of liquid required to trigger this reflex is significantly larger in patients with posterior laryngitis than in healthy controls. Their findings suggest an altered afferent sensory limb of this reflex in patients with posterior laryngitis. Another explanation for pharyngeal reflux may be that a forceful acid reflux event causes abrupt and complete relaxation of the UES.[20,21] Because it occurs independently of swallowing, the larynx is not elevated, the laryngeal outlet is open, and the posterior glottis is in direct contact with the refluxate. However, these mechanisms have not been studied in patients with contact granuloma.

The selection of healthy controls is important in distinguishing between normal and abnormal values in reflux studies. Symptomatic GER is a common disorder; an estimated 7% of adults experience heartburn on a daily basis.[22] In our study, 19 healthy volunteers denied heartburn and acid regurgitation, but 3 of them had pathological pH recordings from the distal esophagus and reflux episodes to the pharynx. Stål et al[23] have shown that hiatus hernia is a common finding in healthy subjects and that it predisposes them to GER. In addition, in their study, symptom evaluation was not sufficient to exclude GER in healthy subjects, and therefore, esophageal abnormalities should be excluded by esophagogastroscopy. Regrettably, the subjects in our study were not examined by endoscopy. Moreover, our healthy controls were on average 20 years younger (mean age, 31.8 years) than the patients (mean age, 51.8 years). However, the impact of age on the degree of distal esophageal reflux differs among previous studies.[24,25] According to Richter et al,[26] there is a trend toward increased reflux with age, but they concluded that for clinical purposes, age did not have an important effect on the physiological parameters of acid reflux. Pharyngeal reflux should, of course, be studied in this respect.

There are sparse normative data concerning pharyngeal reflux events. Koufman[10] had earlier reported that none of 12 normal subjects had any evidence of pharyngeal reflux. Shaker et al[12] studied 12 healthy volunteers, 2 of whom had 1 reflux episode each. In the current study, 3 of the healthy volunteers had 1 pharyngeal reflux episode each, 1 had 2 episodes, and 1 had 8 episodes during the 24-hour registration. The overall number of esophageal reflux episodes has previously proved to be poorly reproducible.[14] Probably, pharyngeal reflux may also be intermittent, and day-to-day variability of these reflux events may account for the discrepancy. That 1 healthy control had 8 pharyngeal reflux events and also pathological esophageal pH monitoring results may suggest that the mere entry of gastric contents into the pharynx does not necessarily result in mucosal injury and development of laryngeal abnormalities.

In summary, increased pharyngeal reflux could be detected in the granuloma group. Furthermore, the percentage of distal esophageal reflux episodes that reached the pharynx was significantly higher in granuloma patients than in asymptomatic controls. In healthy subjects, there were episodes in which refluxed gastric acid reached the pharynx, but they were uncommon.

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SIMILAR COL1A1 EXPRESSION IN FIBROBLASTS FROM SOME PATIENTS WITH CLINICAL OTOSCLEROSIS AND THOSE WITH TYPE I OSTEOGENESIS IMPERFECTA

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Because of the clinical and histopathologic similarities between otosclerosis and type I osteogenesis imperfecta, we examined COL1A1 messenger RNA (mRNA) expression in cultured fibroblasts from patients with clinical otosclerosis to determine whether abnormalities of expression of COL1A1 were present, as has been reported in type I osteogenesis imperfecta. Type I osteogenesis imperfecta has been found to result from mutations in the COL1A1 gene that result in null expression of the mutant allele. Patients with clinical otosclerosis were genotyped for the presence of an expressed 4 base-pair insertion polymorphism in the 3' region of the COL1A1 gene. Skin biopsies were performed, and cultured fibroblast cell lines were established from patients who were heterozygous for the polymorphism. Allelic expression was examined by reverse transcription–polymerase chain reaction and silver-stained polyacrylamide gel electrophoresis. Two of 9 patients with clinical otosclerosis demonstrated null or reduced expression of one COL1A1 allele. The differential expression of the two COL1A1 alleles in all subjects was also examined by a semiquantitative method using an ABI Prism 7700 Sequence Detection System (Taqman). We did this examination to determine whether milder abnormalities in COL1A1 expression might account for the development of otosclerosis in the 7 clinical cases that did not reveal evidence of null expression by the gel technique. Of the same 2 cases of otosclerosis that demonstrated evidence of null expression by gel electrophoresis, both were found to have significant differences in COL1A1 mRNA expression by the Taqman analysis. The remaining 7 cases revealed equal expression of the two COL1A1 alleles similar to that seen in controls. These results suggest that mutations in COL1A1 that are similar to those that occur in type I osteogenesis imperfecta may account for a small percentage of cases of otosclerosis, and that the majority of cases of clinical otosclerosis are related to other genetic abnormalities that have yet to be identified.

KEY WORDS — COL1A1, genetics, mutation, osteogenesis imperfecta, otosclerosis.

INTRODUCTION

Otosclerosis is a bone disease that to the best of our knowledge occurs only within the human otic capsule.[1] It is among the most common causes of acquired hearing loss, with clinical manifestations occurring in approximately 1% of individuals in some populations. Histologic evidence of the disease process can be found in up to 10% of serially sectioned temporal bones. The most common clinical manifestation is a conductive hearing loss that results from stapes fixation. In some cases with large lesions that encroach upon the cochlea, an irreversible sensorineural hearing loss may occur. In most cases, the disease is bilateral.

Histologically, otosclerosis is characterized by a wave of abnormal bone remodeling with resorption of the otic capsule bone and replacement with a hypercellular woven bone that, over time, undergoes further remodeling, resulting in a sclerotic mosaic architecture. Ultrastructural characterization of the cellular components of the active remodeling phase has revealed an absence of acute inflammatory cells and an abundance of histiocytes, osteoblasts, and osteoblast precursor cells, as well as other mononuclear cells.[2,3] Osteoclasts involved in active bone resorption are also present.

At present, the cause of otosclerosis is undetermined. However, there is a well-established hereditary predisposition, with approximately half of all affected individuals having family members known to be affected. Multiple genetic studies of otosclerosis have been reported that support an autosomal dominant mode of inheritance with penetrance in the range of 20% to 40%.[4-8] There is also evidence to suggest that otosclerosis may be heterogenetic, with more than one gene defect giving rise to the otosclerosis phenotype.[9] The genes responsible for the development of otosclerosis have yet to be established. Linkage analysis of a large family from India with presumed otosclerosis has revealed linkage to the 15q25-q26 region of chromosome 15.[10]

We have initiated investigation of the COL1A1

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PCR PRIMER DATA

Primers for Gel or Taqman Analysis	*Primer Sequence 5′ to 3′*	*Size of PCR Product*
18S rRNA A (forward primer)	TTCGGAACTGAGGCCATGAT	101-bp
18S rRNA B (reverse primer)	TTTCGCTCTGGTCCGTCTTG	101-bp
Gel-based analysis (3′ UTR)	CCATGGCTCTTGCAACATCTC	203- or 207-bp
Gel-based analysis (3′ UTR)	CAGCAACACAGTTACACAAGG	203- or 207-bp
Taqman analysis forward primer (no 4-bp insert)	GAGAGTCAGGAAGGGCCACG	120-bp
Taqman analysis forward primer (4-bp insert)	AGTCAGGAAGGGCCAAGCA	124-bp
Taqman analysis reverse primer	GCAACACAGTTACACAAGGAACAGA	

PCR — polymerase chain reaction, rRNA — ribosomal RNA, bp — base-pair, UTR — untranslated region.

gene as one possible candidate gene for otosclerosis. Using association analysis, we found a significant association between clinical otosclerosis and the COL1A1 gene.[11] It is one of the two genes that code for type I collagen, which is the predominant collagen of bone. The other type I collagen gene is COL1A2. Defects in the COL1A1 and COL1A2 genes give rise to osteogenesis imperfecta, one form of which shares a clinical and histopathologic similarity with otosclerosis. Type I or mild osteogenesis imperfecta is most similar to otosclerosis, with temporal bone histopathologic findings indistinguishable from those of otosclerosis, and clinical otologic manifestations that occur in approximately 40% to 50% of affected individuals. As in otosclerosis, the clinical penetrance of otologic manifestations is incomplete. Unlike other forms of osteogenesis imperfecta, most type I osteogenesis imperfecta cases cause very mild skeletal manifestations; many of the patients have normal bone density and no significant history of fractures. A common clinical feature is thin or blue sclerae, which is also seen in some patients with otosclerosis.[12]

Recent studies of type I osteogenesis imperfecta have demonstrated that the majority of cases occur as a result of a mutation in the COL1A1 gene that results in a premature stop codon and null expression of the mutant allele.[13-15] The majority of the COL1A1 messenger RNA (mRNA) that is produced in these individuals comes from the normal allele. The type I collagen that is produced is biochemically and structurally normal, although it is produced in decreased amounts compared to that of normal individuals.

In an effort to determine whether a similar molecular mechanism or genetic mutation is responsible for the development of some cases of otosclerosis, we investigated expression of the COL1A1 gene in individuals with clinical otosclerosis.

METHODS

Messenger RNA and cDNA Selection, Collection, and Preparation. The differential expression techniques described in this article require that each patient's COL1A1 alleles have some difference in their sequence in order to distinguish each allele's contribution to the mRNA pool. We identified 9 individuals with clinical otosclerosis, 4 normal controls, and 2 patients with type I osteogenesis imperfecta who were previously genotyped for heterozygosity at the site of a 4–base-pair (bp) insertion in the 3′ region of the COL1A1 gene. The mRNA from the 2 patients with osteogenesis imperfecta was the generous contribution of Marcia Willing's laboratory at the University of Iowa. To prepare the rest of the samples, fibroblasts from skin biopsies were collected and grown in the tissue culture facility of the Neurogenetics Unit of the Massachusetts General Hospital in Charlestown, Massachusetts.

Approximately 5 × 10^6 cells per sample were processed for total mRNA with Life Technologies' Trizol reagent in accordance with the manufacturer's instructions. The mRNA was quantified on a Genesys 5 Spectronic spectrophotometer, and 1 μg of the total mRNA was removed for processing. Genomic DNA was eliminated by treating the samples with 1 round of DNase I (Gibco) at 37°C for 30 minutes. The DNase was denatured and inactivated by adding 1 μL of 25-mmol/L ethylenediaminetetraacetic acid and heating the sample to 65°C for 10 minutes.

A polymerase chain reaction (PCR) step was performed to test for contaminating genomic DNA as follows: 1 μL of the DNased total complementary DNA (cDNA) was added to 17 μL of PCR Supermix (Gibco) and 1 μL (100 ng/μL) of each of a set of PCR primers that amplifies a 101-bp segment of the 18S ribosomal RNA (rRNA) gene (see Table). The PCR conditions were 2 minutes at 94°C followed by 35 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute and a final 10-minute elongation step at 72°C.

Once the absence of genomic DNA was confirmed, cDNA was generated from the remaining 10-μL sample with the Taqman reverse transcription (RT) reagents kit (Perkin-Elmer, Emeryville, California). The RNA template was mixed with 10 μL of 10×

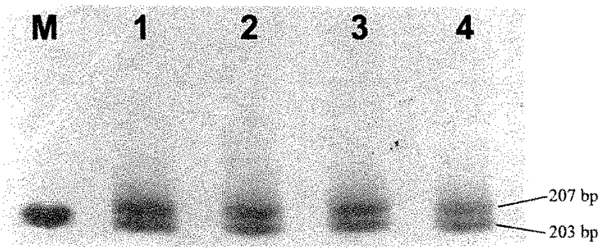

Fig 1. Gel-based analysis shows normal allele expression. Silver-stained gel shows heterozygous COL1A1 alleles in normal control complementary DNA (cDNA) from fibroblasts. Lane M is marker lane. Lanes 1 through 4 show double bands, which represent equal expression of both alleles: 203–base-pair (bp) (bottom) band and 207-bp (top) band.

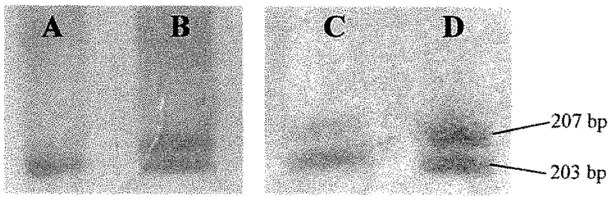

Fig 2. Gel-based analysis shows differential allele expression. Composite photograph of portions of 2 separate silver-stained gels shows COL1A1 alleles of 2 otosclerotic patients who are heterozygous for 4-bp insertion. Lanes A and B are polymerase chain reaction (PCR) results from cDNA and DNA, respectively, of otosclerosis patient L. Lanes C and D are from cDNA and DNA, respectively, of otosclerosis patient V. Note reduced expression of 207-bp (top) band in both cDNAs.

buffer, 22 μL of magnesium chloride, 20 μL of deoxynucleotide triphosphate (dNTP) mix, 5 μL of random hexamer primer, 2 μL of RNase inhibitor, 2.5 μL of MultiScribe reverse transcriptase, and 28.5 μL of RNase-free water for a total volume of 100 μL. The samples were heated to 25°C for 10 minutes, then 48°C for 30 minutes, and finally 95°C for 5 minutes to stop the reaction.

Gel-Based Analysis. We performed a PCR reaction as follows: 1 μL of the cDNA template was mixed with 17 μL of Supermix (Gibco) and 1 μL each of the primers for the COL1A1 3′ untranslated region that flank the polymorphism (see gel-based analysis in Table). The PCR conditions were 2 minutes at 94°C, followed by a variable number of cycles (see below) of 94°C for 1 minute, 56°C for 1 minute, and 72°C for 1 minute. Each PCR reaction was electrophoresed on a 6% denaturing polyacrylamide gel and developed by a silver staining protocol. Since the results were highly dependent on the starting amount of template in each reaction, we had to empirically determine the number of cycles that would give visible differences in the intensity of both the 203-bp and 207-bp fragments. We began each experiment at 35 rounds of PCR and decreased the number of cycles until either one band became less intense than the other or until both bands disappeared together.

Sequence Detector Analysis. Allele-specific oligomers were designed with Perkin-Elmer's Sequence Detection Software to specifically detect only one allele each at the appropriate annealing temperature (see Table; Taqman analysis). In the 7700 Taqman system, the green dye fluorescence signal is measured every few seconds during the PCR cycle as it rises in proportion to the double-stranded DNA generated by PCR.[16,17] Initially, to normalize for differing starting template concentrations, we used the 18S rRNA primers as an endogenous control in the same samples that we tested for COL1A1 (see Table).

All samples were run in triplicate or quadruplicate on the same plate to compensate for pipetting errors. In each well, we used 1 μL of template cDNA, 1 μL of the appropriate primer (100 ng/μL), 14.125 μL of sterile water, 2.5 μL of buffer, 3 μL of magnesium chloride, 2 μL of dNTP mix, 0.25 μL of uracil-*N*-glycosylase, and 0.125 μL of Taq gold. All reagents were from the Perkin-Elmer SYBR green kit (No. 4304886). Amplification was performed at 50°C for 2 minutes, 95°C for 10 minutes, and 40 repetitive cycles of 95°C for 1 minute and 66°C for 1 minute.

RESULTS

Gel-Based Analysis. We observed the relative quantity of the two alleles from each individual sample by visually comparing the apparent differences in the intensities of the bands on a 6% polyacrylamide gel from the same PCR reaction. Because each individual was heterozygous for one copy of the 4-bp insert, as well as a second allele with no insert, both alleles would have to be represented in the PCR reaction as either a 203-bp or a 207-bp fragment. All 4 normal controls demonstrated similar band intensities of the two different COL1A1 alleles (Fig 1). Of the 9 individuals with clinical otosclerosis, only 2 demonstrated evidence of differential mRNA expression of the two different COL1A1 alleles by silver-stained gel electrophoresis (Fig 2, lanes A and C). Seven individuals with clinical otosclerosis had equal band intensities for both alleles, similar to the results shown for the control group in Fig 1 (data not shown).

Sequence Detector Analysis. The cDNA samples were subjected to semiquantitative PCR analysis on the Perkin-Elmer ABI Prism 7700 sequence detector with the green fluorescent dye assay. We were able to measure the cycle number at which the fluorescence intensity of each allele's PCR plot crossed an arbitrary threshold that was set above the fluorescent noise and during the exponential amplification phase in which the signal is least affected by varying reagent concentrations, primer exhaustion, etc. To

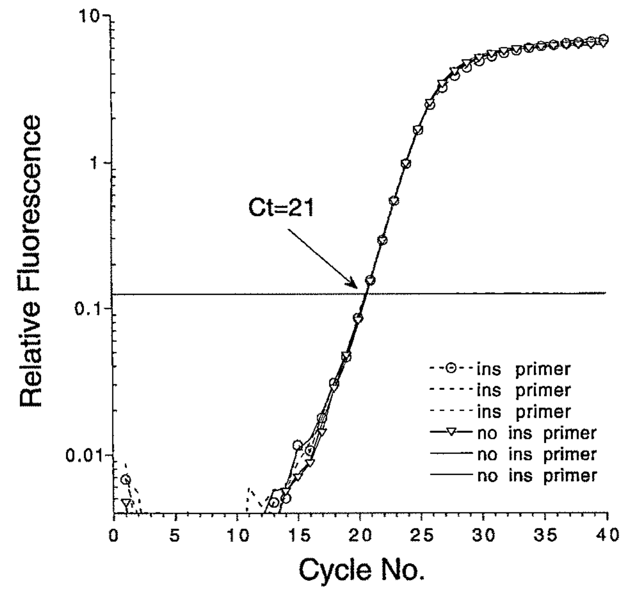

Fig 3. Taqman PCR of both alleles from normal control sample of cDNA. Graphic representation of PCR shows exponential rise in this log plot of increase in fluorescence from both alleles run on same sample of cDNA at same time. Multiple wells of sample are run at same time in order to correct for possible pipetting errors, hence multiple lines on graph. Relative quantity (Ct value) of cDNA is measured at point at which each of curves intersects black horizontal line. Note that both COL1A1 120-bp (triangle) allele and 124-bp (circle) allele overlap in this picture. Their Ct values are same, and there is virtually no difference in relative amounts of COL1A1 messenger RNA (mRNA) produced by either allele in this sample.

compute the relative quantity of mRNA in each allele, we used the comparative ΔΔ threshold cycle (Ct) method.[18] We subsequently determined that our primers gave equal Cts for each allele in the normal controls, and we could observe changes in the expression of all our samples by measuring the threshold cycle of each allele. In this way, we measured relative changes in the expression of the two alleles by the difference between the threshold cycles from each allele by comparing the PCR results using just the "no 4-bp insert" and "4-bp insert" forward primers.

The results of the sequence detector analysis were similar to those of the gel-based analysis and provided a second means of demonstrating what we believed we saw in the gel-based analysis. All normal patients' cDNA and 7 of the 9 otosclerosis patients' cDNA gave results similar to these in Fig 3. Both specimens from individuals with clinical otosclerosis, which revealed evidence of differential expression on gel-based analysis, had quantifiably different levels of COL1A1 allelic expression when measured with the ABI Prism 7700 sequence detector. Otosclerosis patient V exhibited a 3-Ct difference between the two alleles of COL1A1 (Fig 4). Otosclerosis patient L showed no mRNA expression from one allele of COL1A1 (Fig 5). Similar results of differential expression were seen in the 2 individuals with type I osteogenesis imperfecta, 1 of whom displayed a 5-Ct difference in mRNA expression between alleles (Fig 6). The 7 remaining individuals with clinical otosclerosis who did not show any gel-based evidence of differential expression were found to have nearly identical expression of both COL1A1 alleles in the Taqman analysis.

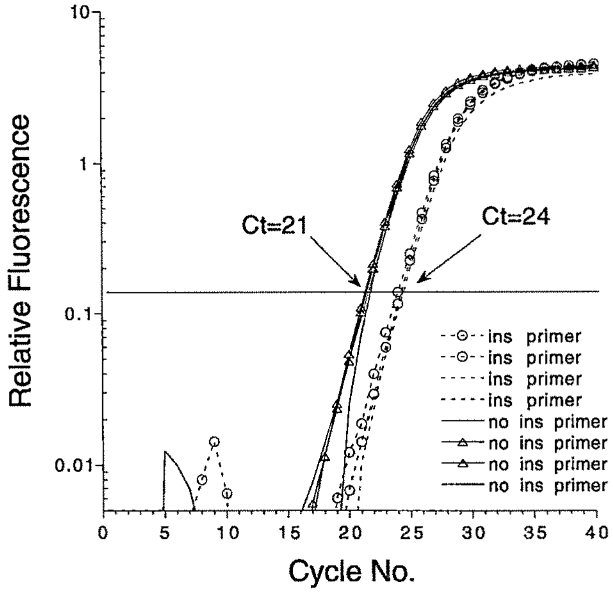

Fig 4. Taqman PCR of cDNA from otosclerosis patient V, who demonstrates differential COL1A1 expression: 124-bp allele (circles) is significantly different from 120-bp (triangles) allele. Difference in relative amounts of cDNA is represented by fact that it takes several more cycles of PCR before 124-bp allele begins to show exponential growth.

DISCUSSION

These results suggest that some cases of clinical otosclerosis may have an underlying genetic cause similar to that of type I osteogenesis imperfecta. One fifth of the specimens from the otosclerosis patients in this experiment demonstrated differential expression of COL1A1 mRNA in a fashion similar to that which has been reported for type I osteogenesis imperfecta. None of the patients with clinical otosclerosis initially included in the study had any other known abnormalities of bone metabolism, skeletal abnormalities, or previous bone fractures. Four of the 9 individuals with clinical otosclerosis had mildly blue sclerae.

A surprising finding in the 2 osteogenesis imperfecta cases was that there appears to be some expression of the second allele and that it is not truly a null mutation or loss of heterozygosity. Since 2 cases of clinical otosclerosis revealed findings in COL1A1 expression similar to those seen in type I osteogenesis imperfecta, we considered the possibility that

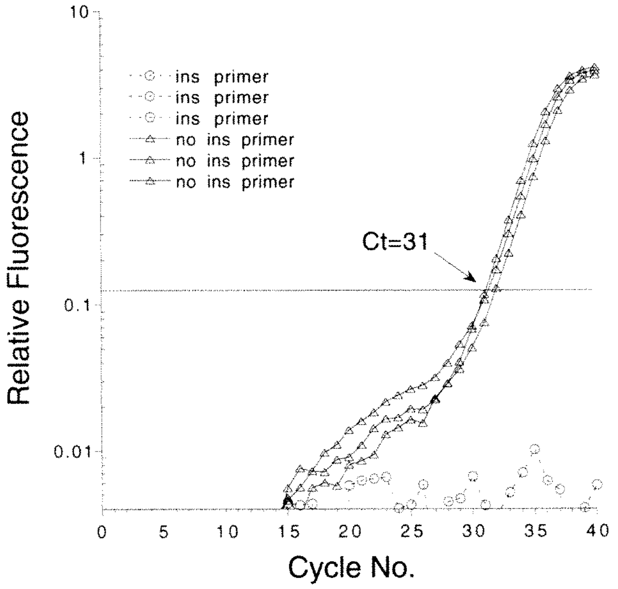

Fig 5. Taqman PCR of cDNA from second patient with otosclerosis (patient L) who also shows differential expression: 120-bp allele (triangles) has Ct of about 31, whereas 124-bp allele signal (circles) never rises out of fluorescent noise at bottom of graph.

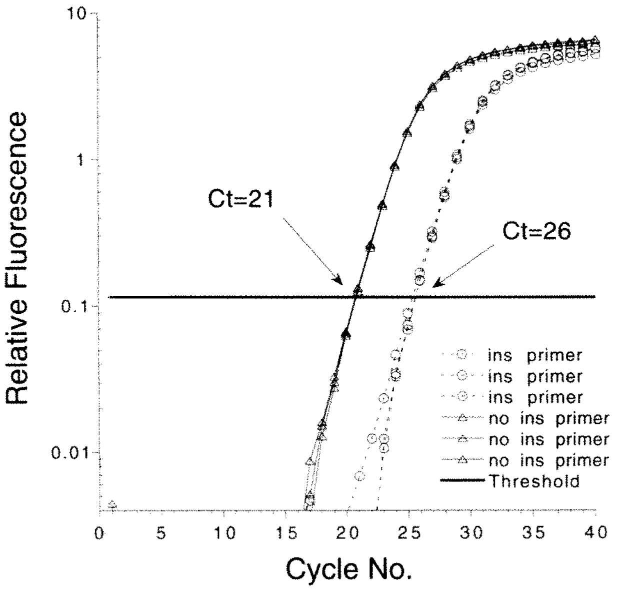

Fig 6. Taqman PCR amplification of two COL1A1 alleles from patient with osteogenesis imperfecta: 124-bp allele (circles) shows reduced expression, with Ct value of about 26 cycles, whereas 120-bp allele (triangles) shows much different value of around 21 cycles. Once again, difference in Ct values between two slopes indicates relative difference in amount of cDNA at start of PCR.

some additional cases may be related to a less-discrepant expression of the 2 COL1A1 alleles and may not be able to be seen by a gel-based method. Since there is evidence that the first intron of the COL1A1 gene plays a critical role in controlling COL1A1 expression and that the binding of specific transcription factors may be affected by alterations in sequence within the first intron, we sought to investigate this potential mechanism by using a more quantitative method for the analysis of COL1A1 expression.[19] The results of the quantitative method using the ABI Prism 7700 sequence detector did not demonstrate evidence of even mild differences in allelic expression between the two different COL1A1 alleles in the 7 affected individuals who showed equal expression of both alleles by the gel technique. However, there is evidence to suggest that the role of the COL1A1 first intron in controlling regulation of the COL1A1 gene is tissue-specific and may be different for bone and skin.[20] There is also evidence that this mode of regulation may be affected by culture conditions.[21] These factors lead us to believe that the possibility of this mechanism has not been resolved with absolute certainty by the above experiments.

Because of the small sample size and a possible selection bias in the acquisition of subjects, no conclusion can be made regarding the frequency of this potential mechanism or of COL1A1 mutations in the overall population of individuals with clinical otosclerosis. Both individuals with clinical otosclerosis who had evidence of differential COL1A1 expression had mildly blue sclerae, and this finding is consistent with an underlying COL1A1 defect. It is possible that a small percentage of patients with clinical otosclerosis have unrecognized or undiagnosed type I osteogenesis imperfecta. Unfortunately, clinical data collected from participants in this study did not include details of patients' dental histories, which might have provided greater insight into this distinct possibility. One of the 2 patients in this study with reduced COL1A1 allelic expression (otosclerosis patient L) developed osteopenia during pregnancy and ultimately received a diagnosis of osteogenesis imperfecta. We are currently in the process of analyzing the DNA of these individuals, as well as others with clinical otosclerosis, for the presence of COL1A1 mutations to confirm and further characterize the nature of the underlying genetic defect. In addition, we are continuing to search for other potential genes and investigate other potential contributing factors that may play a role in the development of otosclerosis.

CONCLUSIONS

A small percentage of cases of clinical otosclerosis may be caused by mutations in the COL1A1 gene that are similar to the mutations that give rise to type I osteogenesis imperfecta and result in null expression of the mutant allele. More subtle differences in COL1A1 allelic expression, which might occur as the result of mutations or polymorphisms within the COL1A1 first intron, do not appear to play a role in the development of otosclerosis.

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SIMPLE ON-LINE ENDOTRACHEAL CUFF PRESSURE RELIEF VALVE

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Ischemic injury of the tracheal mucosa in the endotracheally intubated patient is directly proportional to the tracheal tube cuff pressure. At a cuff pressure of 30 cm H_2O, the tracheal mucosal blood flow becomes partially obstructed, and at a pressure of 45 cm H_2O, the obstruction to the tracheal mucosal blood becomes total, leading to tracheal mucosal damage and subsequent complications. In our institute, we have developed a simple and very inexpensive method to gauge the cuff pressure. We use a regular 20-mL syringe attached in line with the connector of the endotracheal tube cuff. In this manner, we monitored the intracuff pressure in 120 patients who underwent ear or neck surgery. The syringe was connected to the tube cuff and inflated with 15 mL of air. The syringe was left constantly connected to the cuff. In addition, the cuff pressure was measured with the Mallinckrodt Hi-Lo aneroid pressure gauge at the beginning of surgery and hourly thereafter for the duration of surgery. At the same time, a check for leakage around the cuff was made by auscultation with a stethoscope above the sternal notch. Multiple comparisons between the repetitive intracuff pressure measurements revealed that there were no significant differences in the intracuff pressure values measured at the different times of surgery. These results indicate that there was an adequate venting of the excess intracuff pressure and also that there was no leakage around the cuff.

KEY WORDS — cuff pressure, endotracheal tube.

INTRODUCTION

Ischemic injury of the tracheal mucosa in an endotracheally intubated patient is directly proportional to the tracheal tube cuff pressure. Partial obstruction of the tracheal mucosal blood flow commences when the cuff pressure reaches 30 cm H_2O, and obstruction becomes total at a pressure of 45 cm H_2O, leading to tracheal mucosal damage and subsequent long-term sequelae.[1] There is evidence that mucosal damage can occur within hours, and some case reports have even suggested ischemia occurring within 15 to 30 minutes with a cuff pressure above 30 cm H_2O.[2] Cuffs on tubes should be inflated with just enough air or gas to prevent leaks. This practice limits the pressure on the tracheal epithelium so as to prevent damage or even necrosis.

Nitrous oxide is 35 times more soluble than nitrogen in blood and so tends to diffuse into air-containing cavities more rapidly than nitrogen is absorbed into the bloodstream. Therefore, nitrous oxide will diffuse into a cuff that is filled with air. A 50% mixture of nitrous oxide will eventually increase the number of molecules of gas present in the cuff by a factor of two.[3] The rate at which this increase in cuff pressure occurs is dependent upon the permeability of the material from which the cuff is manufactured: plastic is less permeable to nitrous oxide than rubber. Therefore, during anesthesia, nitrous oxide may diffuse into the cuff of the endotracheal tube, causing an increase in the intracuff pressure.[4] Alternatively, filling the cuff with saline solution is reliable but messy and therefore seldom used.[5] Using the anesthetic gas mixture to inflate the cuff maintains a constant pressure only for as long as the gas concentration is kept constant, and should the concentration of nitrous oxide be decreased, the subsequent loss of an airway seal could be dangerous.[6] Therefore, some type of adjustment mechanism for regulation of endotracheal tube cuff pressure to a safe range is necessary. In our institute, we have developed a simple and very inexpensive method to monitor the cuff pressure. We use a regular 20-mL syringe attached in line with the connector of the endotracheal tube cuff.

METHODS

Intracuff pressure was monitored by the suggested method in 120 patients who underwent ear or neck surgery (American Society of Anesthesiologists grade I or II). The mean age of the patients was 42 years (SD, 11 years), and the mean weight was 78.44 kg (SD, 12.30 kg). Anesthesia was induced with intravenous injection of thiopental sodium (4 to 5 mg/kg) and fentanyl citrate (2 µg/kg). Intubation was facilitated by either atracurium besylate (0.5 mg/kg) or vecuronium bromide (0.1 mg/kg). We used a high-volume, low-pressure cuff with an 8- to 8.5-mm in-

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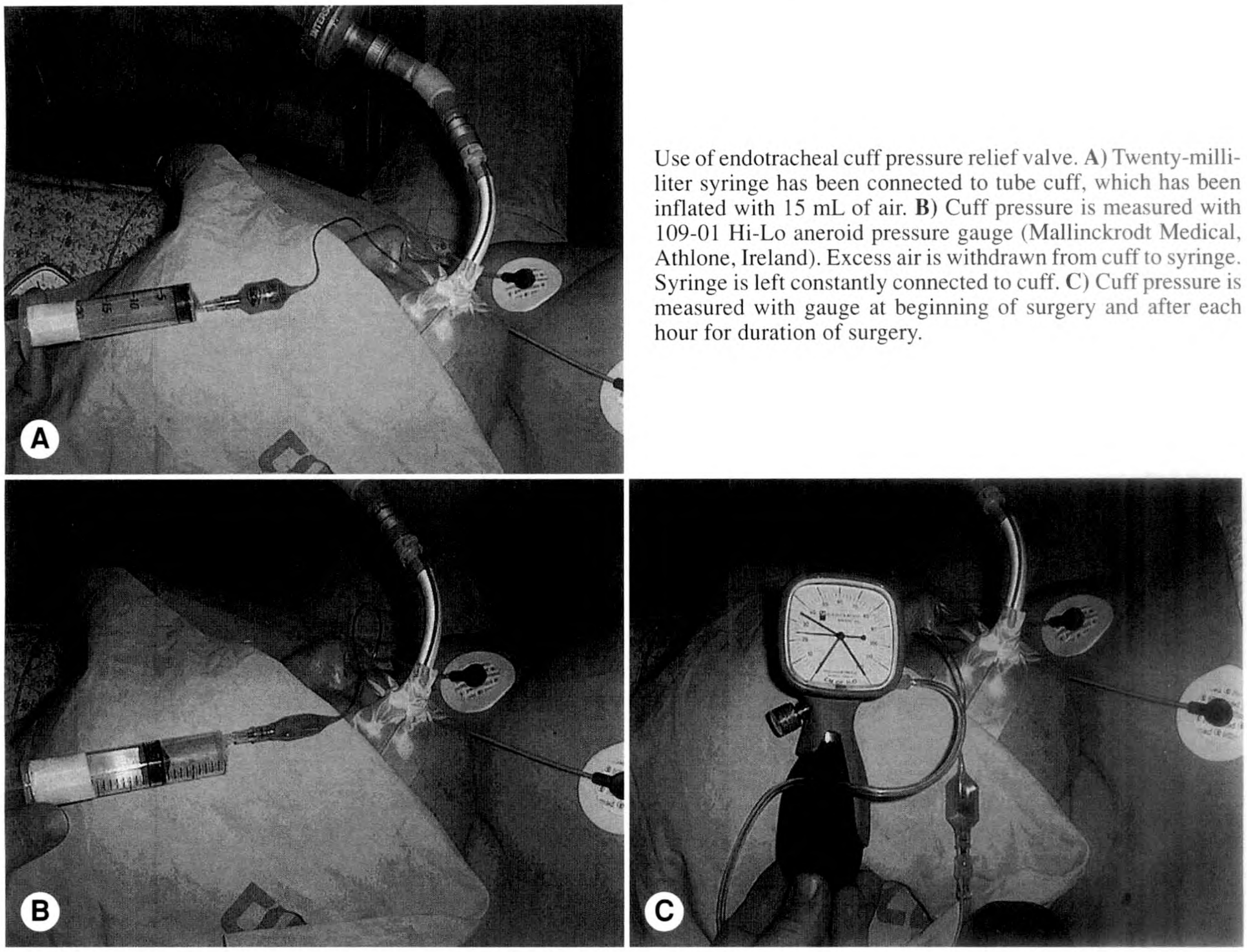

Use of endotracheal cuff pressure relief valve. **A**) Twenty-milliliter syringe has been connected to tube cuff, which has been inflated with 15 mL of air. **B**) Cuff pressure is measured with 109-01 Hi-Lo aneroid pressure gauge (Mallinckrodt Medical, Athlone, Ireland). Excess air is withdrawn from cuff to syringe. Syringe is left constantly connected to cuff. **C**) Cuff pressure is measured with gauge at beginning of surgery and after each hour for duration of surgery.

ternal diameter size for men and a 7.5- to 8-mm size for women (RAE tube, Mallinckrodt Medical, Athlone, Ireland). Anesthesia was maintained by nitrous oxide and oxygen (at a ratio of 50%/50%) with isoflurane (0.5% to 1.5%) as needed, and fentanyl was also given where necessary.

In order to control the pressure of the cuff, we used a 20-mL Terumo syringe (Europe NV, Leuven, Belgium). The syringe was connected to the tube cuff and inflated with 15 mL of air (see Figure, A). If there was any excess pressure, air was withdrawn from the cuff to the syringe (see Figure, B). The syringe was left constantly connected to the cuff. The cuff pressure was measured with the Mallinckrodt 109-01 Hi-Lo aneroid pressure gauge (see Figure, C) at the beginning of surgery (as stated previously) and hourly thereafter for the duration of surgery. A check for leakage around the cuff was performed by auscultation with a stethoscope above the sternal notch in the same time period during which the intracuff pressure was measured.

RESULTS

In the 120 patients, we found the intracuff pressure as measured by the Mallinckrodt manometer to be stable for the entire duration of surgery. The average duration of surgery was 2 hours 48 minutes. The shortest operation took 2 hours, and the longest 3 hours 36 minutes.

Multiple comparisons between the repetitive intracuff pressure measurements (analysis of variance for repeated measures) revealed that there were no significant differences in the intracuff pressure values measured at the different times of surgery. The baseline (after intubation) mean cuff pressure ± SD was 15.6 ± 2.2 cm H_2O. The mean pressures were 15.3 ± 2.3, 15.2 ± 2.3, and 15.4 ± 2.3 cm H_2O at 1 hour, 2 hours, and 3 hours, respectively. These results indicate that there was adequate venting of the excess intracuff pressure and also that there was no leakage around the cuff. This finding was confirmed by auscultation in the suprasternal notch with a stethoscope during inspiration. At the same time, the effectiveness of the mechanical ventilation was ascertained.

DISCUSSION

In order to estimate and consequently maintain an adequate intracuff pressure, most anesthetists use the finger palpation technique and, as a result, often misjudge the inflation pressure.[7] In 1975, Stanley[8] proposed using a simple pressure relief valve. Numerous different methods and apparatus have been suggested for preventing nitrous oxide diffusion into the cuff or for monitoring the intracuff pressure. In 1980, Kim[9] reported use of a tracheal tube cuff stabilizer in order to prevent diffusion of nitrous oxide into the endotracheal cuff and a subsequent increase in intracuff pressure. Fujiwara et al[4] proposed a new gas barrier type of tube that prevented high cuff pressure resulting from diffusion of nitrous oxide. Other authors recommend measuring the intracuff pressure by means of the Mallinckrodt Hi-Lo aneroid pressure gauge. The intracuff pressure is maintained between 20 and 25 cm H_2O by periodically venting the endotracheal tube cuff according to the pressure measured.[1] But these objective pressure devices are rarely a part of the standard anesthetic equipment and are not always available in every operating room, mainly because of their high cost.

In our technique, the syringe described acts as a safety valve to control the excess pressure caused by the diffusion of nitrous oxide into the cuff. This is especially important in head and neck operations, in which the anesthetist does not always have easy access to or direct contact with the patient's head. Nitrous oxide diffusion into the cuff is of real concern, as the subsequent increase in the intracuff pressure may cause ischemic injury to the tracheal mucosa.

CONCLUSIONS

In our experience, constant intracuff pressure monitoring is essential in order to prevent tracheal mucosal injury by high-pressure endotracheal tube cuffs on the one hand and leakage of mechanically ventilated air around underinflated endotracheal tube cuffs on the other hand. Cuff pressure monitoring is of the essence in surgery of long duration and during surgery in which the anesthetist does not have easy access to the patient's head. We propose the use of this syringe as a simple pressure-venting and monitoring device that is reliable, inexpensive, and always available both inside and outside the operating room.

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IMAGING CASE STUDY OF THE MONTH

GLOSSOPHARYNGEAL NEURALGIA ASSOCIATED WITH ABERRANT INTERNAL CAROTID ARTERY IN THE OROPHARYNX

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INTRODUCTION

Glossopharyngeal neuralgia (GPN) is an uncommon cause of facial pain with a relative frequency of about 1% compared to the incidence of trigeminal neuralgia.[1] It is characterized by intermittent, lancinating pain involving the posterior tongue and pharynx, often with radiation to the external auditory canal or to the neck. The cause of GPN is controversial, but it has largely been attributed to vascular compression of the root entry zone of the glossopharyngeal nerve by the posterior inferior cerebellar artery or the vertebral artery.[2,3] Several cases of GPN caused by other disorders such as carcinoma of the tongue[4] and tonsil[5] have also been reported. We report a case of GPN associated with an aberrant internal carotid artery manifesting itself as a pulsatile mass in the oropharynx.

CASE REPORT

A 69-year-old woman was referred to our clinic complaining of a 1-year history of attacks of pain on the right side of the throat. The pain was sharp and stabbing and radiated from the oropharynx to the right submandibular area. The pain was usually triggered by eating or swallowing and had a duration of 30 to 40 seconds. Previously, the patient had been treated for the pain with nonsteroidal anti-inflammatory drugs without benefit. She had a history of arterial hypertension under medical control for 9 years.

On examination, the mucous membrane of the oropharynx was normal in color and character. Palpation of the root of the tongue triggered an episode of pain. A diagnosis of GPN was made. However, a closer and longer inspection of the oropharynx revealed that the right posterior wall of the oropharynx, behind the palatopharyngeal arch, was pulsating in synchrony with the pulse.

Contrast-enhanced computed tomography revealed a great vessel in the right lateral recess of the oropharynx (Fig 1). By magnetic resonance angiography, kinking of the right internal carotid artery was clearly demonstrated at the level of the oropharynx, with impingement on the posterior oropharyngeal wall (Fig 2). No vascular abnormality was found in the intracranial vessels.

On the basis of these findings, GPN caused by compression of the glossopharyngeal nerve by a kinked carotid artery at the level of the oropharynx was strongly suspected, because the glossopharyngeal nerve passes deep in the tonsillar fossa. The fact that infiltration of 0.5% Xylocaine near the aberrant carotid artery in the oropharynx relieved the throat pain for about 2 hours suggested an association between GPN and the kinked carotid artery. We administered carbamazepine (Tegretol) with escalation of the dose up to 600 mg daily. At this dose, carbamazepine induced relief of the pharyngeal pain. Thereafter, the pain was well controlled with carbamazepine at a dosage of 200 mg/d.

DISCUSSION

First described by Weisenberg[6] in 1910, GPN is characterized by paroxysmal pain in the posterior pharynx, tonsillar fossa, and base of the tongue, often with radiation to the external auditory canal or to the neck. It is frequently induced by swallowing and yawning and commonly lasts from a few seconds to a minute. The clinical history of our case is typical for GPN. The pathogenesis of GPN is unknown, but probably involves both peripheral and central mechanisms.[7] In many cases, pain may be caused by vascular compression of the nerve root entry zone by the vertebral artery or the posterior inferior cerebellar artery, resulting in demyelination and ephaptic transmission.[2,3] This hypothesis has served as a rationale

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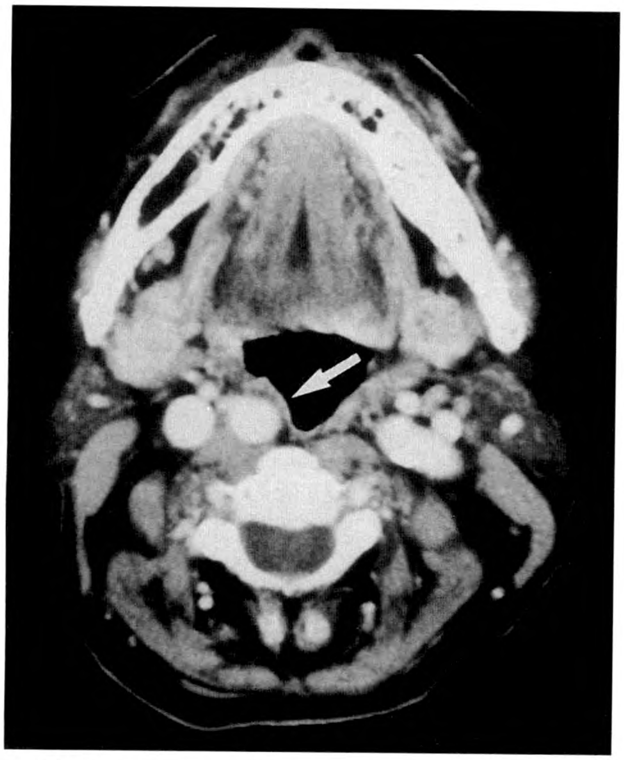

Fig 1. Axial computed tomographic scan shows vascular structure bulging into retropharyngeal area (arrow).

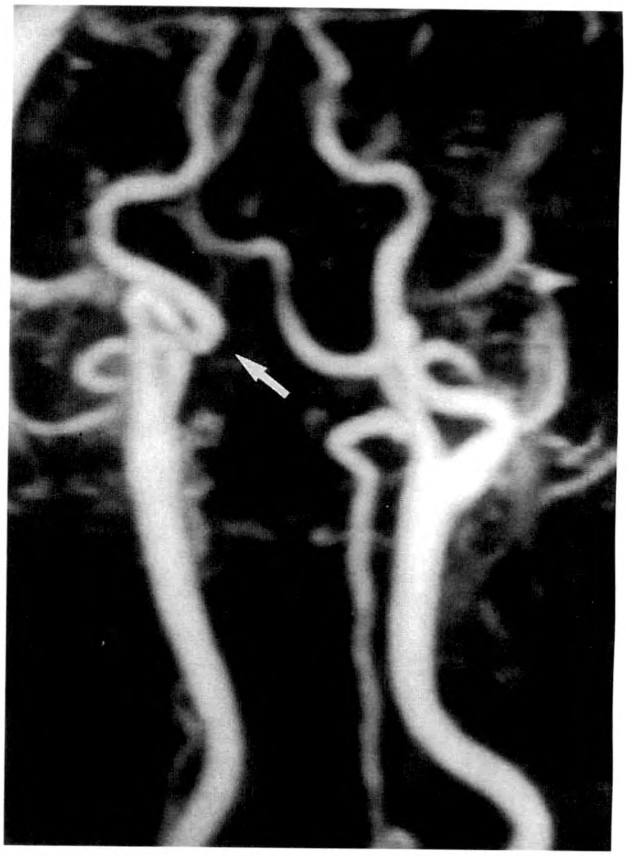

Fig 2. Magnetic resonance angiogram (18° right anterior oblique view) demonstrates kinked right internal carotid artery (arrow) at level of oropharynx.

for microvascular surgical decompression. Alternatively, vascular compression of the nerve may produce repetitive activation of primary afferents in the nerve, which, over time, may produce abnormal hyperactivity and hyperexcitability of the central neurons.[7]

Several cases of GPN secondary to neoplasms such as carcinoma of the tongue[4] and the oropharynx[5] have also been reported. However, there are no previous reports in the literature of GPN associated with an aberrant internal carotid artery in the oropharynx.

Angulation of the internal carotid artery is not a rare condition. Leipzig and Dohrmann[8] divided the variations in the course of the carotid artery into 2 distinct categories: tortuosity and kinking. Elongation, redundancy, undulation, and an S-shaped curve are classified as tortuosity, while any sharp bend in the vessel, as observed in our patient, is classified as kinking. The main causes of these malformations are atherosclerosis and congenital deformity, and the mean age at diagnosis is 58.8 years. Patients are usually asymptomatic, and these malformations are considered relatively benign conditions, although a kink of the internal carotid artery may be associated with cerebrovascular disease. Of greater relevance to otolaryngologists is the importance of recognizing this condition before performing surgical procedures in the pharynx, especially tonsillectomy or adenoidectomy. Several authors have reported fatal hemorrhage after surgery in the presence of this anomaly when undetected.[9]

We considered that GPN in our case was caused by vascular compression of the glossopharyngeal nerve by a kinked internal carotid artery in the oropharynx. The pathogenic mechanism can be traced to the close proximity between the glossopharyngeal nerve and the internal carotid artery at the level of the oropharynx. Some tortuosity or kinking of the internal carotid artery in the oropharynx can compress the glossopharyngeal nerve, as vertebral or posterior inferior cerebellar arteries compress nerves intracranially. In our case, magnetic resonance angiography revealed kinking of the right internal carotid artery at the level of the oropharynx without an intracranial vascular abnormality. The fact that application of Xylocaine near the kinked carotid artery temporarily relieved the pain attacks might support our hypothesis.

Several alternatives, medical and surgical, have been proposed for treatment of GPN.[7] As for treatment with medication, carbamazepine and/or phenytoin have been successful in controlling episodic pain in GPN,[1] as in trigeminal neuralgia. Ketamine, a blocker of *N*-methyl-D-aspartate receptors, has also been reported to be effective in a case of GPN.[7] Our case showed a good response to carbamazepine. When medication fails to relieve the pain, or is contraindicated, surgical treatment may be proposed. The surgical alternatives include resection of the glossopharyngeal nerve extracranially or intracranially, sometimes with resection of the upper rootlets of the vagus nerve.[1,10] But a sense of dryness of the mouth, an irritating cough, and mild difficulty in swallowing are not uncommon postoperative disabilities. Furthermore, the recurrence of pain is possible, especially after extracranial section of the nerve. Microvascular decompression of the glossopharyngeal nerve is advocated as the most appropriate treatment,[2] and seems logical if neurovascular compression is suspected. To date, microvascular decompression at the root entry zone of the glossopharyngeal nerve has been done in many cases of GPN; the immediate and long-term relief of pain has been excellent, and complications are infrequent.[2,3] Because GPN in our case was considered to be associated with an aberrant internal carotid artery in the oropharynx, it might have been possible to relieve the pain by vascular decompression at the level of the oropharynx.

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BOOK REVIEWS

Diseases of the Larynx

Alfio Ferlito, editor. Hard cover, illustrated, indexed, 860 pages, 2000. Arnold, London, England.

Diseases of the Larynx is one of the most comprehensive textbooks ever written for laryngology. This work, which is published at the beginning of the 21st century, is a great tribute to the enduring career of the editor, who is the primary author, demonstrating both hindsight and foresight. It is reminiscent of the compendia of Solis Cohen (1800s) and Jackson (1900s). This multi-authored book is well organized and is easy to read. The chapters on laryngeal development and experimental laryngeal carcinogenesis are particularly noteworthy. The wise selection of chapters representing the spectrum of diseases and disorders that present in the larynx is a tribute to the editor's experience and perspective. There is strength in the coverage of pediatric and adult problems, as well as benign and malignant neoplasia. A minor shortcoming of the book is that there appears to have been a considerable amount of production time between the contributors' completion of the chapters and publication, which has variable significance, depending on the subject matter. This was most evident in surgical technique chapters because of rapid worldwide advancements. Overall, the editor has done an outstanding job at compiling a massive body of information from a highly skilled group of co-authors. *Diseases of the Larynx* is an invaluable laryngological reference and should be in the library of any clinician involved with laryngeal disease.

STEVEN M. ZEITELS, MD
Boston, Massachusetts

Otologic Surgery

Derald E. Brackmann, Clough Shelton, Moisés A. Arriaga, editors. Second edition. Soft cover, illustrated, indexed, 680 pages, 2001. Saunders, Philadelphia, Pa, $175.

Purpose: To communicate the ideas, instruments, and surgical techniques that have evolved in this expanded field of otology and neuro-otology since the publication of the first edition in 1994.

Contents: There are 63 chapters, all dealing, of course, with surgery. Two of the chapters, on otologic instrumentation and intraoperative neurophysiologic monitoring, are so closely related to surgical procedures that they are an integral part of such a book.

Attributes and Limitations: The 3 reasons the editors convey for publishing this second edition are 1) numerous changes in the field of otology, 2) the surgical techniques that have evolved, and 3) the expansion in the field with inclusion of skull base surgery. Indeed, most advances in skull base surgery were introduced within the past 50 years, so this field is a recent phenomenon that has seen many changes. It is obvious from various chapters of this book that the skull base team works in a much-advanced environment that is technically more involved than that of our colleagues of 25 years ago. The field of skull base surgery has (besides William F. House, whose imagination and ingenuity revolutionized this "new" specialty by establishing various neuro-otological procedures) predecessors that brought about the development of the subspecialty. I would include Harvey William Cushing (1869-1939) because of his monograph that depicts specific types of tumors arising in the cerebellopontine angle; Walter E. Dandy (1886-1946) for ventriculography that diagnosed skull base lesions; and Charles Frazier (1870-1930), who pioneered the subtemporal approach to the gasserian ganglion. There are others.

Obviously, the editors gave the authors specific guidelines that were, for the most part, adhered to. This has resulted in a clear and concise treatment of each chapter within the text. It has a straightforward and readable style. There is no basic philosophy of any one author. Several authors may give their management of a particular procedure (eg, tympanoplasty), thus giving the reader a complete overview of how that procedure can be handled. Impressively, there is an unbiased view of various surgical techniques.

So, why the need for a revised edition of *Otologic Surgery* after 7 years? I think it was time for an update, although I am not sure that there are that many new procedures to talk about. The most evident catalyst to be portrayed as a new procedure is the perfusion technique of treating inner ear dizziness, as in Meniere's disease. The current volume elaborates on the chemical treatment of the labyrinth with streptomycin and gentamicin, and omits, except for some basic information on corticosteroids, the dexamethasone treatment of intratympanic labyrinthine disorders. Although this is a very updated book, the matter of dexamethasone perfusion was too recent to meet the publication deadlines of this book (personal communication with one editor). I noticed that many of the authors throughout the text give an otologic instrumentation and procedure description that makes the opening chapter on otologic instrumentation to some extent redundant.

Applicability: The text provides an excellent practical guide and reference source on otologic surgery and is most useful to residents and the seasoned surgeon.

DENNIS G. PAPPAS, MD
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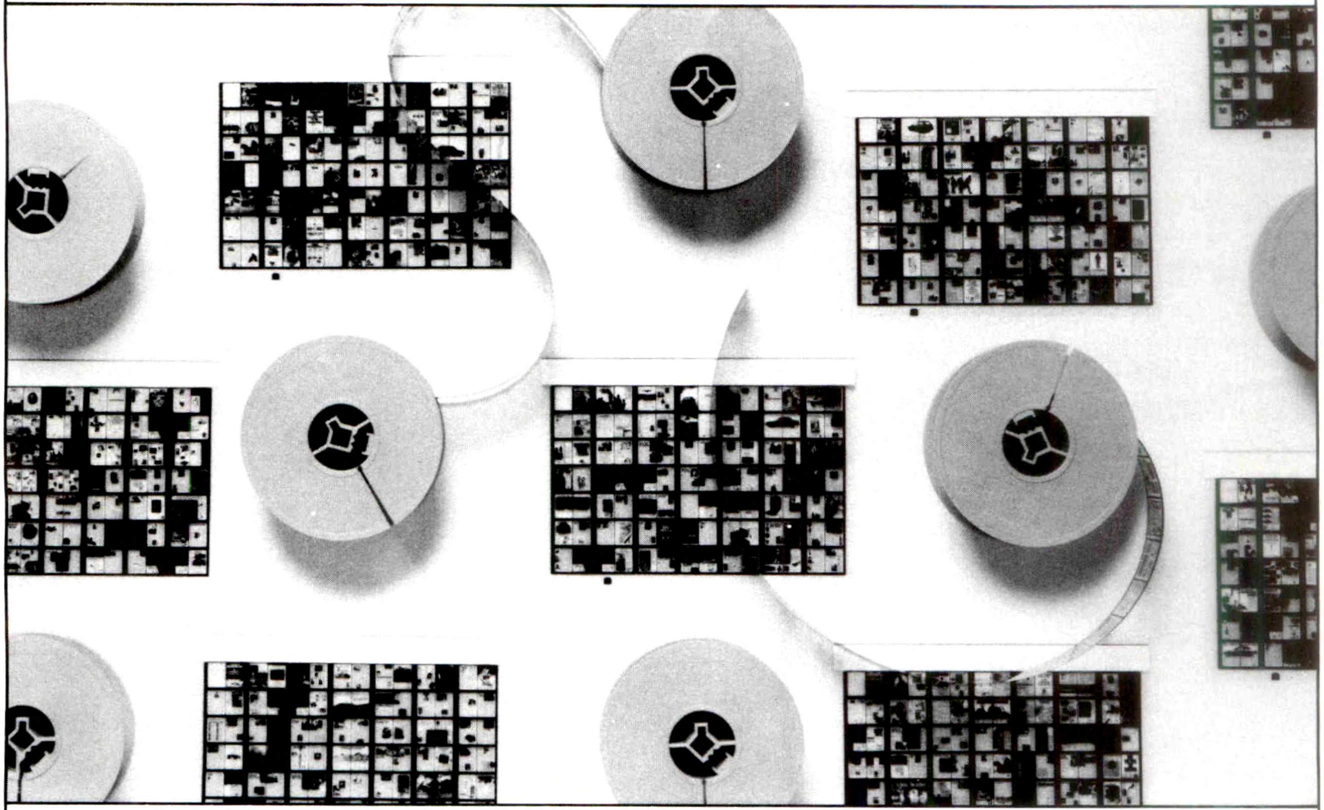

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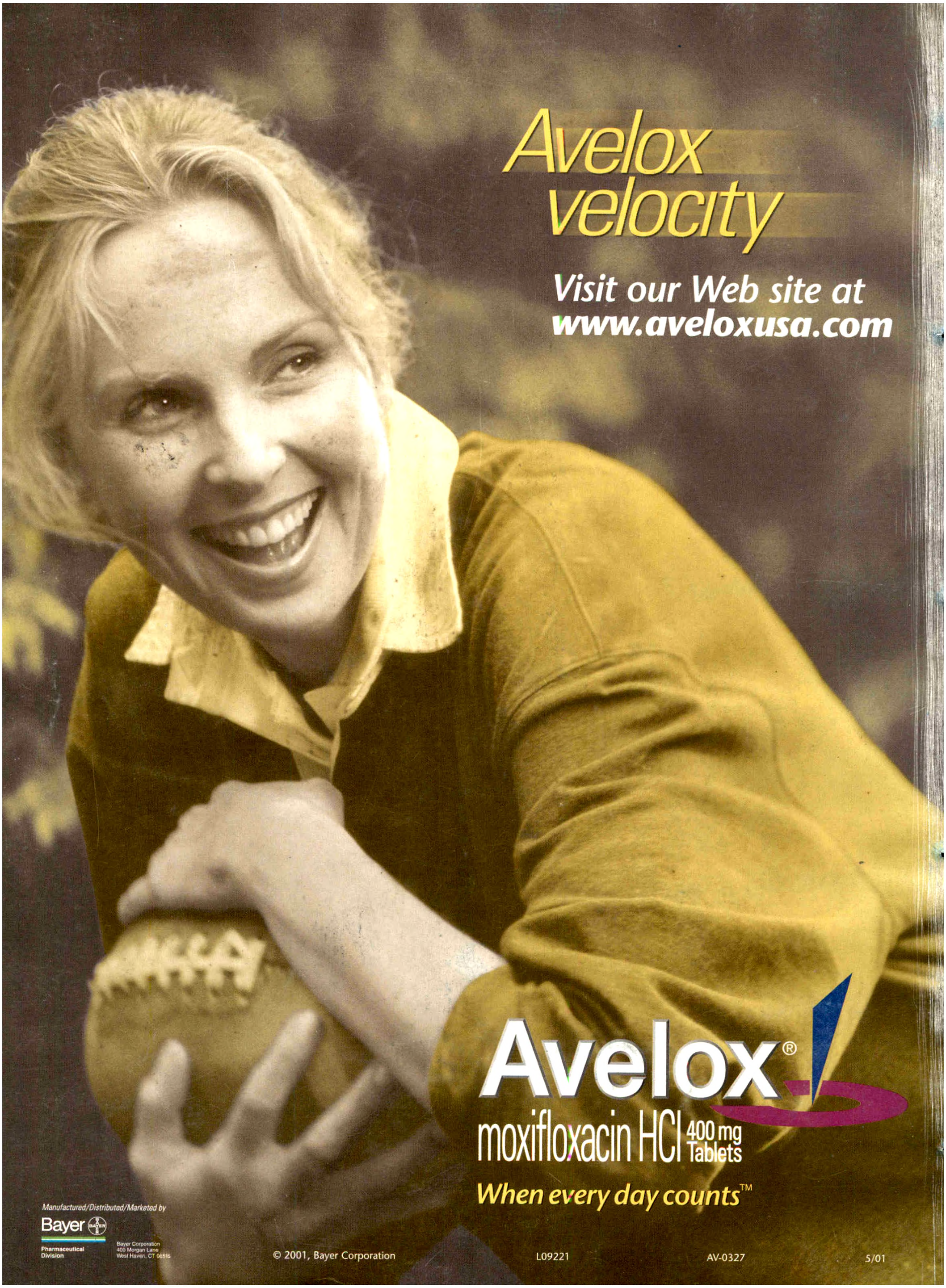

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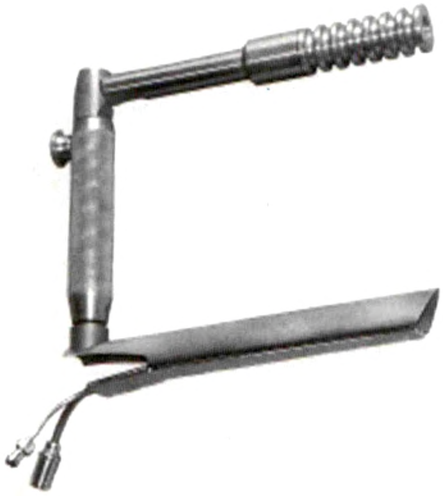

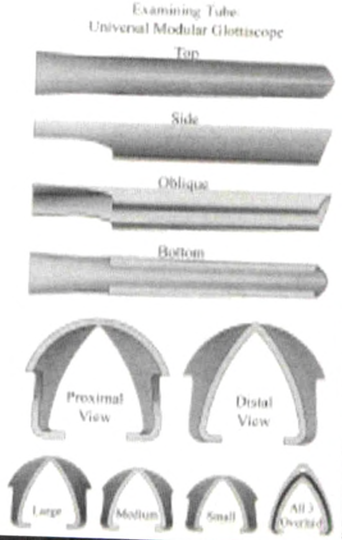

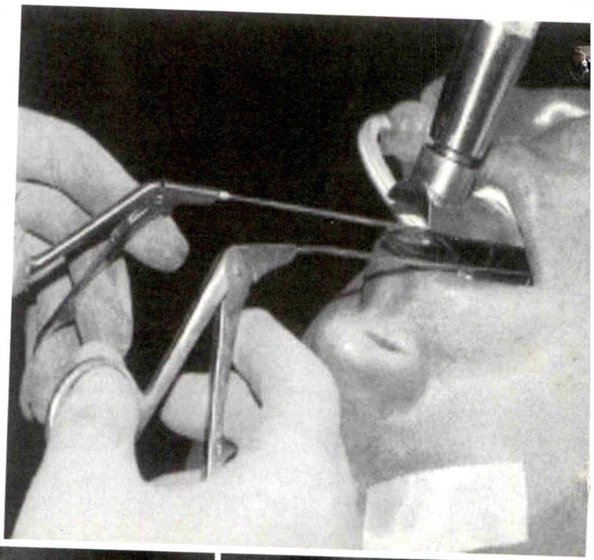

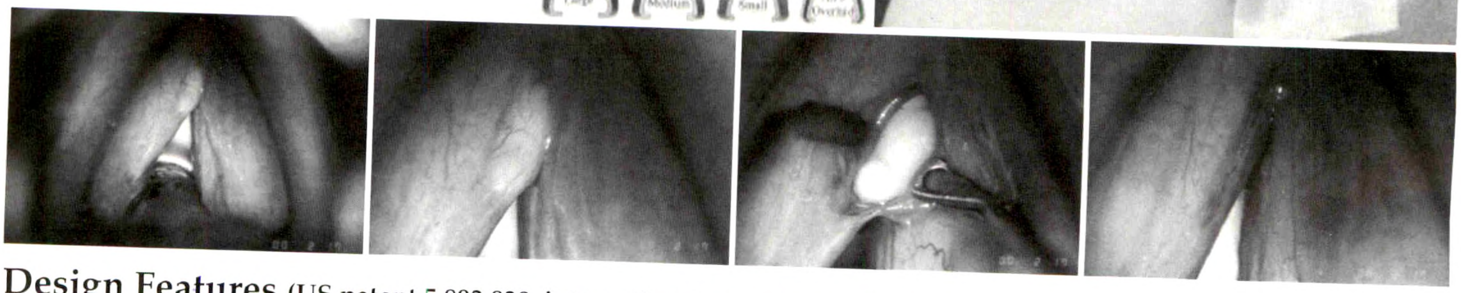

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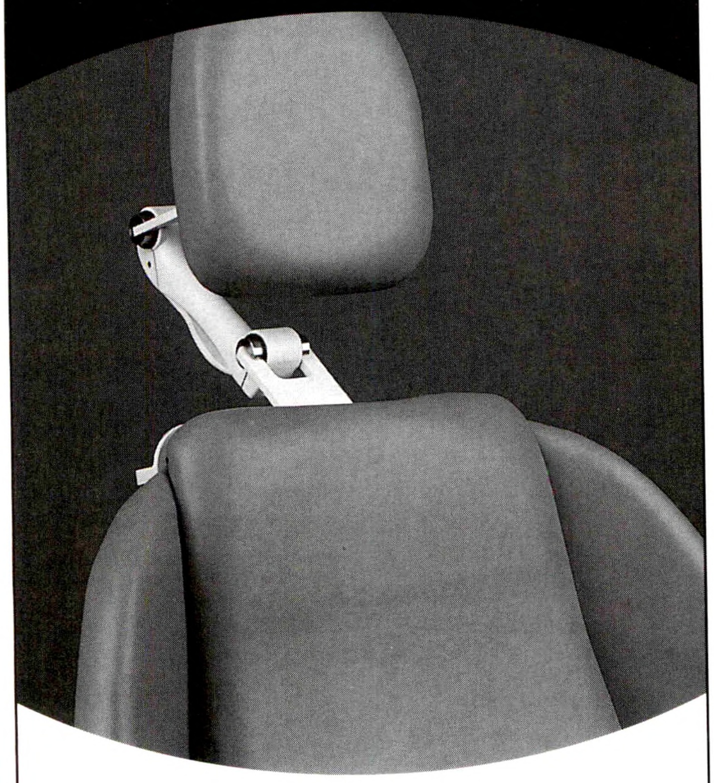

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MEMBRANOUS TRACHEAL RUPTURE IN CHILDREN FOLLOWING MINOR BLUNT CERVICAL TRAUMA

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Injuries to the tracheobronchial tree are well-recognized sequelae of massive blunt or penetrating injuries of the neck or chest. They may also occur as a rare complication of endotracheal intubation. We present 2 cases of a less well-recognized clinical entity, rupture of the membranous trachea following minimal blunt trauma to the neck in children. The case histories and management of this disorder are discussed. Recognition and treatment of this problem requires a high index of suspicion for the lesion and timely investigations. Open repair of the trachea to secure a stable airway is recommended for this injury, unless the wound is small and the wound edges are well approximated.

KEY WORDS — rupture, trachea, trauma.

INTRODUCTION

Injuries to the tracheobronchial tree are well-recognized sequelae of massive blunt trauma or penetrating injuries of the neck or chest in both adults[1-6] and children.[7-11] Membranous tracheal rupture (MTR) has also been described as a rare complication of endotracheal intubation.[12-15] However, a less well-recognized clinical entity is MTR following minimal blunt trauma to the neck. A small series of children with MTR following minimal blunt neck trauma has been previously reported,[11] with conservative management advocated.

We have encountered 2 children with MTR following relatively minor blunt neck trauma, treated at the Royal Children's Hospital, Melbourne, Australia, between 1997 and 2000. We present their clinical course, the investigations and treatment performed, and our philosophy of management of this rare but significant clinical entity.

CASE REPORTS

Case 1. A 29-month-old boy fell from his tricycle onto a wooden bench and struck the anterior part of his neck, sustaining a blunt injury. He quickly developed respiratory distress and facial and neck swelling; he was taken to the emergency room of his local hospital, given inhaled nebulized epinephrine for airway distress, and transferred to a nearby regional hospital. His breath sounds were equal and his saturation levels were adequate, but he had extensive subcutaneous emphysema and audible stridor. There was no evidence of pneumothorax. He was taken to the operating room for stabilization of his airway and assessment of the extent of his injuries 3 hours after his original injury.

In the operating room, the patient underwent laryngoscopy and bronchoscopy, which showed a normal larynx and vocal cords, but extensive edema and blood in the proximal trachea obscuring the lumen. A 2.5-mm–internal diameter endotracheal tube was the only size that could be passed beyond the injured area, but with this in place, his oxygen saturation levels were low, and the tube was changed over a guide wire to a larger tube. However, he could not be ventilated through this larger tube, and remained hypoxic and developed sinus bradycardia. An adequate airway could not be established with a rigid bronchoscope. Cardiopulmonary resuscitation was instituted along with bilateral 18-gauge needle decompression of the thorax, although no pneumothoraces were found. An emergency tracheotomy was performed with a 5.5-mm–inner diameter endotracheal tube inserted into the distal trachea, resulting in improvement in ventilation and restoration of cardiac output. The endotracheal tube was secured, and the patient was observed in the intensive care unit and transferred to our pediatric tertiary care center by air ambulance the following morning.

Upon arrival at our institution, the patient was transferred to the operating room for assessment and repair of his airway. Rigid bronchoscopy revealed a vertical linear laceration of the membranous trachea from immediately below the cricoid cartilage, just left of the midline, extending to approximately the fifth tracheal ring. The wound edges were separated by the esophagus prolapsing into the tracheal lumen. Esophagoscopy did not reveal any mucosal disruption. The neck was opened and the trachea was di-

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vided in the anterior midline from the inferior border of the cricoid ring to the tracheotomy site. The posterior trachea was repaired with interrupted 4-0 Vicryl sutures, and the anterior trachea was similarly closed, with a tracheotomy tube replaced in a more inferior position through a separate tracheal opening. The patient had an uncomplicated recovery, and elective bronchoscopy performed 9 days after the operation showed good healing of the injured area but some suprastomal granulation tissue and mild tracheal collapse. He was discharged home 15 days after the injury with the tracheotomy tube in situ. Decannulation was initially delayed because of a documented bacterial pneumonia and a later respiratory syncytial virus infection, but the patient was successfully decannulated 2 months after his injury. He had no airway symptoms and a normal voice at follow-up 3 years after the injury.

Case 2. A 5$^1/_2$-year-old boy tripped while walking and struck the anterior part of his neck on an open drawer. He had immediate neck pain and quickly developed significant neck swelling and an altered voice. He had no penetrating wounds. He was seen at his local hospital 50 minutes after the injury and was found to have subcutaneous emphysema of the neck, face, and upper chest, but did not have stridor or respiratory distress. He was transferred to the intensive care unit of our hospital and was stable, with findings as described above. Cervical spine radiographs suggested a possible subluxation of C2 on C3, although this could not be distinguished from a variant of normal. A chest radiograph was normal. He was taken to the operating room for rigid bronchoscopy, where anesthesia was induced with an inhalational agent, and then maintained by spontaneous ventilation. Bronchoscopy revealed a linear tear in the membranous trachea, with wide separation of the wound edges, which extended from the lower edge of the cricoid cartilage inferiorly for about 4 to 5 cm. Esophagoscopy did not reveal any mucosal disruption.

The patient was intubated, and the trachea was exposed via a horizontal neck incision and opened with a midline vertical incision from the lower border of the cricoid cartilage inferiorly until the entire laceration was in view. The endotracheal tube was replaced in the lower end of the tracheotomy incision. The laceration was repaired with interrupted 4-0 Vicryl sutures, and the patient was reintubated nasally. The anterior trachea was closed with interrupted 4-0 Vicryl sutures, and the neck skin was closed over a Penrose drain. The patient was returned to the intensive care unit, and the nasal endotracheal tube was removed uneventfully 3 days after the operation. A Philadelphia neck collar was kept in place for several weeks after the operation because of concern about a slight subluxation of C2 on C3 that was found on the cervical spine radiographs, but there were no clinical signs of neurologic impairment. The patient was discharged from the hospital 11 days after the injury with a normal airway and normal voice, tolerating a full diet. He was asymptomatic at his 1-month follow-up.

DISCUSSION

Longitudinal rupture of the membranous trachea following relatively innocuous blunt trauma to the neck is a rare clinical entity that has only been described in the pediatric population. Nonetheless, an injury to the anterior part of the neck that would seem minimal can produce a tracheal rupture, as demonstrated by our patients and the previous report in the literature.[11] Subcutaneous emphysema, the classic hallmark of tracheal injury, was present in both of our patients and in 3 similar patients presented by Hager et al.[11] Any child with a clinical suspicion of a tracheal rupture, no matter how trivial the neck injury may seem on history, must be managed with the prospect of an unstable airway in mind. Securing the airway safely is the primary objective, and this is best achieved when the possibility of an MTR is borne in mind, and adequate equipment, anesthetic, and surgical expertise are available. Difficulties encountered with securing the airway in the primary management of case 1 presumably reflected a lack of appreciation of this condition, with likely passage of the endotracheal tube through the ruptured trachea into the mediastinum.

The literature describes a large population of patients, particularly adults, with significant tracheal or bronchial injuries after major trauma, especially motor vehicle accidents. These high-energy injuries can clearly transmit enough force to the tracheobronchial tree to cause rupture or avulsion of the airway.[1-10] However, in the 2 cases we describe, the forces involved were much smaller. Studies of the trachea have concluded that the membranous trachea is most subject to rupture, as it is the weakest point of the laryngotracheal complex.[16] The U-shaped rings of cartilage strengthen the anterior and lateral walls. The exact mechanism of rupture of the membranous trachea following minor trauma in our series is less clear. Presumably, the raised intratracheal pressure following a brief compressive force, coupled with a closed glottis, are sufficient to exceed the tensile strength of the membranous trachea. Another significant factor is the contribution of the direct blunt trauma to the anterior trachea, which would compress the U-shaped cartilage rings onto the firm cervical spine. This mechanism would explain why tracheal rupture has tended to occur at the exact level of the trauma.[16] In the pediatric population, the cartilaginous rings are more flexible and less likely to fracture than in adults. These rings can distort considerably, forcing the ends of the U-shaped rings apart and directly tearing the mucosa of the membranous trachea in a longitudinal fashion.

Air escaping through a tracheal rupture will produce considerable subcutaneous emphysema, as seen in both of our patients. The first of our cases emphasizes the potential for life-threatening airway instability. Thus, these injuries must be evaluated and treated expeditiously in a controlled setting. We recommend prompt laryngoscopy and bronchoscopy under spontaneous ventilation, which prevents exacerbation of the emphysema. A rigid ventilating bronchoscope also aids in securing a compromised airway and could be left in situ as an alternative to an endotracheal tube while the trachea is being exposed and opened. Evaluation of the esophagus to rule out concurrent injury is mandatory after securing of the airway. Although computed tomographic scanning can sometimes suggest the diagnosis,[17] it should not take the place of direct evaluation of the airway. Of course, as with any trauma situation, evaluation of the cervical spine and chest with radiographs is essential before any intervention, particularly in cases in which the neck may be flexed during operation.

Small ruptures of the trachea either from trauma or as a consequence of endotracheal intubation have been managed in the past with conservative therapy.[11,13,15] However, in a series of 3 pediatric patients with similar mechanisms of injury treated conservatively,[11] the lengths of the tracheal injuries were much shorter than those found in our patients. In that series, lesions of 1 and 1.5 cm were treated conservatively, and a 3-cm laceration was treated with fibrin glue. None of the injuries showed any gaping of the tracheal wounds. With tracheal ruptures of any significant size, particularly if there is any gaping of the wound edges as demonstrated in our 2 patients, definitive open repair seems to be the most prudent course of action. This will ensure a stable airway and prevent worsening of the subcutaneous emphysema, as well as minimize the risk for contamination of the neck and mediastinum with airway pathogens. In the past, a left cervicotomy has been described,[18] but this open approach has been avoided by many, citing risks to the recurrent laryngeal nerves.[11] We used an anterior approach through the anterior wall of the trachea, as has been described by Mussi et al.[12] This is the approach that has been well established in pediatric otolaryngology for laryngotracheoplasty in the treatment of subglottic stenosis. We believe that this affords complete evaluation of the tracheal injury, which can sometimes be underestimated on endoscopy. The postoperative management is analogous to that of single-stage laryngotracheoplasty, with a brief period of endotracheal intubation. Tracheotomy is generally unnecessary.

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COMPARISON OF THE MICROBIAL COMPOSITION OF VOICE PROSTHESIS BIOFILMS FROM PATIENTS REQUIRING FREQUENT VERSUS INFREQUENT REPLACEMENT

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This study was performed to establish a possible difference in biofilm composition in patients who require frequent versus infrequent prosthesis replacement. Only Groningen button voice prostheses that were removed because of increased airflow resistance or leakage of food or liquids through the prosthesis were considered for this study. These prostheses were selected from a total of 692 failed voice prostheses over a 2-year evaluation period. The failed voice prostheses were subdivided into a short-lifetime group, corresponding to an implantation period of less than 4 months (20 voice prostheses), and an extended-lifetime group, corresponding to an implantation period of greater than 9 months (18 voice prostheses). The biofilm was removed from the valve sides of the prostheses. The bacterial strain *Rothia dentocariosa* and the yeast strains *Candida albicans* I and *Candida tropicalis* were the predominant strains isolated from the biofilms on the voice prostheses in the short-lifetime group, whereas in the extended-lifetime group, *R dentocariosa* was found with a fourfold lower isolation frequency and *C albicans* I was found with a twofold lower isolation frequency. *Candida tropicalis* was absent from the extended-lifetime group.

KEY WORDS — biofilm, *Candida*, identification, *Rothia dentocariosa*, *Staphylococcus*, *Streptococcus*, voice prosthesis.

INTRODUCTION

Biomedical implants, such as indwelling silicone rubber voice prostheses,[1] prosthetic heart valves,[2] indwelling urinary catheters,[2] silicone breast implants,[3] and prosthetic joints,[2] have contributed to the solution of several serious medical problems. Although the development of these biomaterial implants can be considered successful, a drawback of the introduction of biomaterial implants into the human body is that microorganisms have the tendency to adhere to biomaterial surfaces and cause biomaterial-related infections. Adhering microorganisms are organized within a biofilm, a unique environment in which they are protected against host defense mechanisms and influences of antimicrobial agents, which is critical to their survival. Consequently, biomaterial-related infections inevitably lead to replacement or removal of an implant.

Although several studies have identified the strains and species involved in voice prosthetic biofilms, microbial analyses have not yet been aimed toward establishing a difference in biofilm composition between patients who require frequent prosthesis replacement and those who require infrequent prosthesis replacement. The aim of this study was to evaluate whether differences exist between silicone rubber voice prostheses that have failed within 4 months and prostheses that have failed after 9 months from the time of insertion, in terms of microbial composition of the biofilm.

STUDY DESIGN

Over a 2-year evaluation period, 128 different patients visited the outpatient clinic of the Department of Otorhinolaryngology of the University Hospital of Groningen with a total of 692 failed voice prostheses, which were all retrieved after replacement. Subsequently, we considered for this study only Groningen button voice prostheses that were removed because of increased airflow resistance that hampered tracheoesophageal speech or because of leakage of food or liquids through the prosthesis. These voice prostheses were subdivided into 2 arbitrarily defined lifetime groups, representing extremes. Accordingly, 20 prostheses were entered in the short-lifetime group (18 men, 2 women; mean age, 63.5 ± 10.6 years), corresponding to an implantation period of less than 4 months, and 18 prostheses were included in the extended-lifetime group (15 men, 3 women; mean age, 67.6 ± 8.2 years), corresponding to an implantation period of greater than 9 months.

The explanted prostheses were directly transferred

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YEASTS AND BACTERIA ISOLATED FROM VOICE PROSTHESES SUBDIVIDED ACCORDING TO LIFETIME

	Isolation Frequency (%)	
	Short Lifetime	*Extended Lifetime*
Yeast species		
Candida albicans I	70	44
Candida humicola	10	17
Candida glabrata	10	17
Candida tropicalis	20	0
Candida parapsilosis	0	17
Saccharomyces cerevisiae	0	17
Candida krusei	10	6
Trichosporon cutaneum	0	11
Bacteria or genera species		
Rothia dentocariosa	45	11
Streptococcus	25	17
Streptococcus vestibularis	5	6
Streptococcus cricetus	0	6
Streptococcus suis	0	6
Streptococcus salivarius	5	0
Streptococcus mitis	5	0
Streptococcus pyogenes	5	0
Staphylococcus	25	11
Staphylococcus epidermidis	10	0
Staphylococcus aureus	5	0
Staphylococcus lugdunensis	5	0
Staphylococcus warneri	5	0
Staphylococcus cohnii	0	6
Staphylococcus intermedius	0	6
Escherichia coli	5	6
Stomatococcus mucilaginosus	0	6
CDC group EF4	10	6
Photobacterium logei	10	0
Pediococcus pentosaceus	0	6
Kocuria kristinae	5	0
Leuconostoc mesenteroides	5	11
Bacillus laevolacticus	0	6

Short-lifetime group (life of prostheses < 4 months) comprised 20 voice prostheses; extended-lifetime group (life of prostheses > 9 months) comprised 18 voice prostheses. Isolation frequency is defined as number of Groningen button voice prostheses on which given strain was found relative to total number of Groningen button voice prostheses in group.

to 60-mL plastic containers filled with reduced transport fluid (sodium chloride 0.9 g/L, ammonium sulfate 0.9 g/L, potassium dihydrogen phosphate 0.45 g/L, magnesium sulfate 0.19 g/L, dipotassium hydrogen phosphate 0.45 g/L, disodium ethylenediaminetetraacetic acid 0.37 g/L, L-cysteine hydrochloride 0.2 g/L, pH 6.8) for microbial analyses.

All explanted prostheses were macroscopically examined. Subsequently, the biofilm was removed from the valve sides of the prostheses by scraping, and after sonication for 60 seconds, the isolated microorganisms were serially diluted in reduced transport fluid. Subsequently, the microorganisms were plated on brain-heart infusion (Oxoid, Basingstoke, England) agar plates for yeasts and blood agar plates for bacteria. After 3 days at 37°C in an aerobic incubator, different yeast and bacterial colonies were distinguished on the basis of visual inspection and subcultured.

Once pure fungal and bacterial cultures were obtained on a plate, the yeast strains were identified by the ID 32 C identification system (BioMérieux, Marcy-l'Etoile, France), and bacteria were first examined with light microscopy and Gram staining. Subsequently, the bacterial strains were identified by the GP MicroPlate (Biolog, Hayward, California) for gram-positive bacteria and by the GN MicroPlate (Biolog) for gram-negative bacteria.

RESULTS

The explanted voice prostheses showed an enormous variety in macroscopically visible deterioration of the silicone rubber on the valve side. Within both the short- and extended-lifetime groups, large brown deposits were seen on the esophageal side of some prostheses, but prostheses with a macroscopically clean surface were also retrieved.

The isolation of the microorganisms colonizing the 20 Groningen button voice prostheses of the short-lifetime group resulted in a collection of 49 bacterial and 27 yeast isolates. Gram staining showed that 36 bacterial isolates were gram-positive. The biofilms on the 18 Groningen button voice prostheses in the extended-lifetime group included 25 yeast isolates and 43 bacterial isolates, of which 30 turned out to be gram-positive.

The Table shows the frequency of isolation of the identified strains in the short- and extended-lifetime groups. The 52 yeast strains were mainly *Candida* species (47 isolates); other strains identified in the extended-lifetime group were *Saccharomyces cerevisiae* (3 isolates) and *Trichosporon cutaneum* (2 isolates). Among the *Candida* strains, the most conspicuous difference between the short- and extended-lifetime groups concerned *Candida albicans* I, *Candida tropicalis*, and *Candida krusei*, which were all found more frequently in the short-lifetime group than in the extended-lifetime group. *Candida humicola*, *Candida glabrata*, *Candida parapsilosis*, *S cerevisiae*, and *T cutaneum* were more frequently isolated in the extended-lifetime group.

Most of the bacterial strains could be identified as *Rothia dentocariosa* (11 isolates), streptococci (8 isolates), and staphylococci (7 isolates). Particularly, *R* *dentocariosa* and staphylococci were isolated more frequently in the short-lifetime group than in the extended-lifetime group. The numbers of streptococci isolated in the two groups did not differ in a meaningful way. Apart from the above strains, several other bacterial strains that belonged to different genera were occasionally isolated (see Table). The identification system used could not classify 51 of the bacterial isolates, which were generally rod-shaped organisms (76%).

DISCUSSION

Clinically, an impressive interindividual variation exists with regard to the lifetime of indwelling voice prostheses in laryngectomized patients. This extreme range in prosthesis lifetimes among patients is reflected in a recent study on the lifetimes of Provox voice prostheses, reported to be on average 115 days, with a range of 7 to 583 days.[4] Sometimes, for unknown reasons, patients can switch from the group needing frequent replacement to the group needing replacement once a year, or vice versa, without evident changes in medication or lifestyle. The causative factors for these extreme differences in prosthesis lifetimes are not entirely known, but it has been suggested that irradiation dose, volume of irradiated salivary gland tissue, residual salivary flow rate, time passed after irradiation or insertion of a prosthesis, surgical or drug therapy, and prosthetic tooth replacement are involved.

All potential causes suggested above contribute to a disturbance of the oral microflora of a laryngectomized patient, which may stimulate oral pathogens such as yeasts to become dominant, as known, for instance, in denture stomatitis.[5] Brown et al[6] studied the influence of xerostomia after radiotherapy on human oral microflora and found, together with the saliva shutdown, a shift toward cariogenic microorganisms in the oral cavity after irradiation — in particular, increases in *Streptococcus mutans*, *C albicans*, and species of *Staphylococcus* and *Lactobacillus*.

In the present study, we demonstrated a difference in the microbial composition of biofilms on indwelling silicone rubber voice prostheses that failed within 4 months or after 9 months from the time of insertion.

Most of the microorganisms identified were yeast and bacterial strains that are normally present on the skin and in the oral cavity. Furthermore, a few bacterial strains originating from food and dairy products were identified. As in previous studies,[7-9] the most common microbial strain colonizing the valve side of the voice prostheses was *C albicans*. In the short-lifetime group, 70% of the voice prostheses retrieved were colonized with *C albicans* I, whereas in the extended-lifetime group, the frequency of *C albicans* I isolation was only 44%. *Candida tropicalis* was only identified in the short-lifetime group (20%), and never in the extended-lifetime group. Van der Mei et al[10] demonstrated in vitro that *C albicans* and *C tropicalis* both can be stimulated to grow into silicone rubber. Already, within 14 days, the initial biodeterioration of the silicone rubber by these yeast strains could be shown by scanning electron microscopy. This property of yeasts to grow into the silicone rubber of voice prostheses provides them with additional protection against environmental attacks,[11,12] next to the protection offered by the biofilm mode of growth. In a longitudinal study by Neu et al[13] involving ESKA-Herrmann prostheses, it was found that *C albicans* is predominantly present in biofilms during the first 8 days after insertion, whereas *C tropicalis* was only isolated from biofilms on voice prostheses more than 8 days after insertion. A major difference between the present study and the one by Neu et al, however, is that here, we only considered prostheses removed for clinical reasons, whereas Neu et al replaced prostheses at fixed points in time to demonstrate a possible colonization sequence in the absence of clinical reasons for replacement.

In this study, *R dentocariosa* was the most frequently isolated bacterial strain in the short-lifetime group — a finding suggesting its association with prosthesis failure. Staphylococci also were most frequently isolated in the short-lifetime group. Interestingly, Palmer et al[7] found that 50% of Blom-Singer prostheses that failed within 60 days were colonized by *S aureus*, whereas in the present study, *S aureus* was identified only once in the short-lifetime group. Palmer et al, however, studied Blom-Singer valves, which can be cleaned daily by the patient, whereas cleaning of Groningen button voice prostheses by patients is not usual. Furthermore, Palmer et al studied the biofilm of the complete Blom-Singer prosthetic valve, including the shaft and the tracheal side, which might well be colonized by strains and species different from those on the valve side of a voice prosthesis, as examined in the present study. Ell et al[14] demonstrated an association between *Candida* in voice prosthetic biofilms and failure due to increased airflow resistance of valves after use for less than 75 days. Also, Ell et al[15] found that valve leakage was associated with the prevalence of streptococci in biofilms in the valve and that enterococci in biofilms on the esophageal side were associated with high airflow resistance failure of voice prostheses within 75 days of use.

It has been suggested that the adhesion of bacteria to voice prostheses is a prerequisite for subsequent colonization by yeasts.[13] Such a sequence in colonization by bacteria and yeasts has been described also for denture stomatitis.[5] Millsap et al[16] studied in vitro adhesion of yeasts suspended in saliva to silicone rubber with and without adhering bacteria present on the silicone and demonstrated that most bacterial strains suppressed adhesion of yeasts. However, *C albicans* and *C tropicalis* adhered in higher numbers to silicone rubber when adhering *R dentocariosa* or *S aureus* was present. It could be concluded that adhesive interactions between these bacterial and yeast strains play a crucial role in the development of a biofilm. This conclusion is supported here by the observation that *R dentocariosa* was the predominant strain colonizing prostheses in the short-lifetime group. The role suggested by Millsap et al[16] for *S aureus* in stimulating adhesion of yeasts is in line with the association between *S aureus* prevalence and prosthesis failure reported by Palmer et al.[7] As *R dentocariosa* and *S aureus* seem to emerge as bacterial strains that stimulate colonization of voice prostheses by yeasts that then lead to their failure, exclusion of these two bacterial strains from the oral microflora by selected antibiotics or salivary peptides might well be more effective than the currently applied antimycotic regimen, which has no proven clinical efficacy.[17]

From the results of this study, it can be concluded that *C albicans* I, *C tropicalis*, and *R dentocariosa* play a significant role in the early failure of Groningen button silicone rubber voice prostheses. Furthermore, it is of interest that the retrieval protocol, the type of voice prosthesis, and the areas sampled on retrieved voice prostheses all have an impact on the microbial strains and species identified in the biofilms formed on the prostheses.

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METACHRONOUS SECOND PRIMARY CANCERS AFTER SUCCESSFUL PARTIAL LARYNGECTOMY FOR INVASIVE SQUAMOUS CELL CARCINOMA OF THE TRUE VOCAL CORD

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The current retrospective studies documented the incidence, sites of occurrence, risk factors, and outcome of metachronous second primary cancers (MSPCs) among an inception cohort of 410 patients with invasive squamous cell carcinoma of the true vocal cord successfully treated with partial laryngectomy at a single institution. The Kaplan-Meier actuarial life-table method was used to document the relationship between the incidence of MSPCs and survival data. Univariate analysis was performed for potential statistical relationships among the incidence of MSPCs, the site of occurrence, and different variables. The overall incidence of MSPCs was 23.9% (98/410). The 10-year actuarial survival estimate for MSPCs was 20.4%.The incidence curve of MSPCs was linear, resulting in a 2%/y rate of development for MSPCs. In univariate analysis, the only variable that demonstrated a statistical correlation with the incidence of MSPCs was smoking, with MSPCs being statistically more likely to occur in smokers than in nonsmokers ($p = .04$). The main sites of origin for MSPCs were the lung (25.5% of cases), other non–upper aerodigestive tract sites (32.7%), and the upper aerodigestive tract (41.8%). The 10-year actuarial estimates for MSPCs were 9.1% in the upper aerodigestive tract, 7.1% in sites other than the lung or upper aerodigestive tract, and 6.6% in the lung. The incidence curve for MSPCs was linear, whatever the site of origin, resulting in 1%/y, 0.7%/y, and 0.6%/y rates of development for MSPCs in the upper aerodigestive tract, sites other than the lung or upper aerodigestive tract, and the lung, respectively. Survival was statistically reduced when an MSPC developed; the 10-year actuarial survival estimates were 76.8% in patients who did not develop an MSPC and 43.7% in patients who developed an MSPC ($p < .0001$). Overall, 68.4% of patients who developed an MSPC (67/98) died of this disease. The 10-year actuarial survival estimates were 24% for lung MSPCs, 43.7% for non-lung, non–upper aerodigestive tract MSPCs, and 63.4% for upper aerodigestive tract MSPCs.

KEY WORDS — glottic carcinoma, metachronous second primary cancer.

INTRODUCTION

Partial laryngectomy and radiotherapy are the conventional conservative treatment modalities in selected patients with invasive squamous cell carcinoma of the true vocal cord. In such patients, the local control rates are high.[1-4] However, in patients with laryngeal carcinoma, metachronous second primary cancers (MSPCs) are increasingly responsible for disease-specific mortality.[5-9] A few reports have documented and analyzed the incidence of MSPCs in patients with carcinoma of the true vocal cord managed with radiotherapy,[5-9] but to our knowledge (Medline search), no data based on a large series of patients with invasive squamous cell carcinoma of the true vocal cord successfully treated with partial laryngectomy and a long duration of follow-up have yet been reported. This lack of data was the initial impetus for the current retrospective study based on an inception cohort of 410 patients with invasive squamous cell carcinoma of the true vocal cord successfully treated with partial laryngectomy at a single institution. More specifically, our report was designed to document the incidence, site of occurrence, risk factors, and outcome of MSPCs after the successful treatment of invasive squamous cell carcinoma of the true vocal cord with partial laryngectomy.

MATERIALS AND METHODS

The current studies were based on the analysis of the medical files, operative charts, and pathological reports of 410 patients with an isolated and previously untreated invasive squamous cell carcinoma of the true vocal cord without a palpable jugulocarotid lymph node in whom partial laryngectomy, performed in our department between August 1963 and August 1989, resulted in definitive control of the tumor.

Patients managed with radiotherapy alone; with tumors considered as transglottic, infraglottic, or supraglottic in origin; with tumors originating from the anterior commissure; with noninvasive squamous cell

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TABLE 1. UNIVARIATE ANALYSIS FOR POTENTIAL STATISTICAL RELATIONSHIP BETWEEN DEVELOPMENT OF MSPC AND DIFFERENT FACTORS

	Patients With MSPC	*Patients Without MSPC*	*p*
Gender			.74
Male	95	298	
Female	3	14	
Age (y)			.25
Range	36-82	29-90	
Mean	60	60	
T stage of glottic tumor			.95
T1	68	218	
T2	26	80	
T3 and T4	4	14	
Alcohol habit (403 patients)			.51
No alcohol intake	18	71	
Alcohol intake	76	237	
Alcohol consumption (g/L per day)			.63
1-40	44	126	
41-80	26	83	
80	6	28	
Tobacco habit (403 patients)			.04
Nonsmokers	7	48	
Smokers	88	260	
Tobacco intake (pack-years)			.17
Range	3-90	1-100	
Mean	35	34	
Charlson comorbidity index[14]			.48
0	58	194	
1	30	84	
2	6	28	
3 to 5	4	6	
Induction chemotherapy			.44
Yes	27	74	
No	71	238	

χ^2 Test and Mann-Whitney U test were used for qualitative variables analysis and quantitative variables analysis, respectively.

MSPC — metachronous second primary cancer.

carcinoma of the true vocal cord; with a palpable jugulocarotid lymph node; with metastatic disease; with a synchronous second primary tumor; or who failed the initial partial laryngectomy either locally at a lymph node site or at a distant site did not take part in the current retrospective study.

There were 393 male patients and 17 female patients with a mean age of 60 years (range, 29 to 90 years). According to the 1997 American Joint Commitee on Cancer staging classification system,[10] the initial invasive squamous cell carcinoma of the true vocal cord was classified as T1 in 286 patients, T2 in 106 patients, T3 in 17 patients, and T4 in 1 patient. The invasive squamous cell carcinoma of the true vocal cord was moderately to well differentiated in 400 patients and verrucous in 10 patients. The partial laryngectomy was an endoscopic laser resection in 2 patients, a vertical partial laryngectomy in 316 patients, and a supracricoid partial laryngectomy in 92 patients. In the current series, very few patients were treated with endoscopic laser resection, as this treatment option was introduced at our institution in the very late 1980s. Preoperative or postoperative radiotherapy of the larynx was not used. One hundred one patients (24.6%) had an induction chemotherapy regimen before the completion of the partial laryngectomy. The rationale and results for using an induction chemotherapy regimen before the partial laryngectomy have already been reported[11] and will not be discussed in the current article.

Follow-up data were collected during periodic visits to our clinic, which were made once a month during the first 6 postoperative months, every 2 to 3 months during the second year, every 3 to 4 months during the third year, every 6 months during the fourth and fifth years, and every year thereafter. Yearly chest radiography was performed. Routine endoscopy and barium swallow esophagography were not part of this follow-up unless they were indicated by symptoms and/or clinical examination. Other physicians and the patients were contacted for additional information if adequate follow-up data were not available in our records. Three hundred eighty-eight patients (94.7%) were followed for a minimum of 10 years or until death. The MSPCs were identified according to the criteria of Warren and Gates.[12] The second primary cancer was considered as metachronous if it occurred at least 6 months after the initial carcinoma of the true vocal cord. An MSPC in the supraglottic and/or infraglottic larynx was defined as a completely separate tumor arising outside the margins of resection of the initial carcinoma of the true vocal cord. An MSPC at the glottic level was defined as a completely separate tumor arising outside the margins of resection of the initial carcinoma of the true vocal cord more than 5 years after the completion of the partial laryngectomy. A lung carcinoma was considered an MSPC if it was solitary and presented in a patient who did not have local and/or nodal recurrence. Statistical analysis of the incidence of MSPCs and survival data was based on the product-limit method of Kaplan and Meier,[13] and the log-rank test method was used for statistical comparison. Univariate analysis was also performed for a potential correlation of different factors to the incidence of MSPCs. The factors under analysis were gender, age, the Charlson comorbidity index,[14] a tobacco habit (smokers versus nonsmokers), the amount of tobacco intake (pack-years), an alcohol habit

TABLE 2. SITE OF ORIGIN AND PATHOLOGICAL TYPE OF NINETY-EIGHT MSPCs

			Pathological Type			
	Patients		*Squamous*		*Nonsquamous*	
Site of Origin	*No.*	*%*	*No.*	*%*	*No.*	*%*
Upper aerodigestive tract carcinomas						
Larynx	17	17.3	17	17.7		
Oropharynx	9	9.2	9	9.2		
Hypopharynx	6	6.1	6	6.1		
Oral cavity	3	3.0	3	3.0		
Esophagus	5	5.1	5	5.1		
Cervical lymph node with unknown primary	1	1.0	1	1.0		
Lung carcinomas	25	25.5	18	18.4	7	7
Non-lung, non–upper aerodigestive tract carcinomas						
Gastrointestinal tract	9	9.2			9	9.2
Prostate gland	8	8.1			8	8.1
Liver	4	4.0			4	4
Hematopoietic system	3	3.0			3	3
Brain	1	1.0			1	1
Pancreas	1	1.0			1	1
Breast	1	1.0			1	1
Bladder	1	1.0	1	1		
Kidney	1	1.0			1	1
Skin	1	1.0	1	1		
Thyroid gland	1	1.0			1	1
Metastatic disease (second primary not individualized)	1	1.0			1	1
Total	98	100	61	62.3	37	37.7

(drinkers versus nondrinkers), the amount of alcohol consumption (intake in grams of alcohol per liter ingested per day) at the time of partial laryngectomy, the T classification of the true vocal cord carcinoma, and the use of an induction chemotherapy regimen before partial laryngectomy (Table 1[14]). The parametric χ^2 test and the nonparametric Mann-Whitney U test were used for analysis of qualitative variables and quantitative variables, respectively. Significance was assumed if the p value was .05 or less.

RESULTS

The overall crude incidence of MSPCs was 23.9% (98/410). The 10-year actuarial survival estimate for the development of MSPCs was 20.4%. The incidence curve of MSPCs was linear, resulting in a 2%/y rate for development of MSPCs. The only variable that demonstrated a statistical correlation with the development of MSPCs was smoking, with the development of MSPCs being more frequent in smokers than in nonsmokers (Table 1; $p = .04$), but the degree of tobacco intake among smokers did not increase the risk of the development of MSPCs (Table 1; $p = .17$). Eight patients had a third primary malignancy. The sites of origin of the third primary malignancy were the oropharynx (1 patient), the sinuses of the face (1), the skin (1), the kidney (1), the gastrointestinal tract (1), the liver (1), and the hematopoietic system (2).

The overall crude distribution of the MSPCs by site is shown in Table 2. According to the sites of origin of the MSPCs, 3 populations were distinguished: the upper aerodigestive tract (larynx, hypopharynx, oropharynx, oral cavity, and esophagus) in 41.8% of cases (41/98), sites other than the lung or upper aerodigestive tract in 32.7% of cases (32/98), and the lung in 25.5% of cases (25/98; Table 2). The 10-year actuarial survival estimates for the development of MSPCs were 9.1% in the upper aerodigestive tract, 7.1% in sites other than the lung or upper aerodigestive tract, and 6.6% in the lung. The incidence curve of MSPCs was linear, whatever the site of origin, resulting in 1%/y, 0.7%/y, and 0.6%/y rates of development for the upper aerodigestive tract, sites other than the lung or upper aerodigestive tract, and the lung, respectively.

The 10-year actuarial survival estimates were 43.7% for patients who developed an MSPC and 76.8% for patients who did not develop an MSPC. According to the log-rank test, the risk of death was 2.5 times higher in patients who developed an MSPC than in patients who did not develop an MSPC ($p < .0001$). Overall, 68.4% of patients who developed an MSPC (67/98) died of this disease.

The 10-year actuarial survival estimates were 24% for patients with lung MSPCs, 43.7% for patients with non-lung, non–upper aerodigestive tract MSPCs, and 63.4% for patients with upper aerodigestive tract MSPCs. According to the log-rank test, the risk of death was 2.3 times higher in patients with lung MSPCs than in patients with upper aerodigestive tract or non-lung, non–upper aerodigestive tract MSPCs ($p = .014$).

DISCUSSION

According to the criteria of Warren and Gates,[12] the overall crude incidence of MSPCs was 23.9% (98/410) in our series. This figure was in line with the overall 22% crude incidence of MSPCs reported by Fujita et al[6] in a series of 158 patients with T1 glottic cancer treated with radiotherapy alone. In our series, the classification of the MSPCs according to site of origin was 25.5% for the lung, 32.7% for sites other than the lung or upper aerodigestive tract, and 41.8% for the upper aerodigestive tract (Table 2). As compared to the data of Fujita et al (39% in the lung, 28% in the upper aerodigestive tract, and 33% in sites other than the lung or upper aerodigestive tract), our figures suggested a higher overall crude incidence of upper aerodigestive tract MSPCs and a lower overall crude incidence of lung MSPCs. Such a difference might be explained by the minimum 10-year follow-up achieved in 94.7% of patients in the current series (as compared with a minimum follow-up of 12 months in the series by Fujita et al) and by the exclusion from our series of patients who had local or nodal failure after the initial conservative treatment, as either form of failure might increase the risk of subsequent development of a distant metastasis (which, when occurring in the lung, might be confused with a lung MSPC).

By the Kaplan-Meier[13] actuarial life-table method, the 10-year actuarial survival estimate for the development of MSPCs was 20.4% in our series. The incidence curve of MSPCs was linear, resulting in a 2%/y rate of development for MSPCs. The incidence curve was linear whatever the site of origin of the MSPC, resulting in 1%/y, 0.7%/y, and 0.6%/y rates of development for MSPCs in the upper aerodigestive tract, sites other than the lung or upper aerodigestive tract, and the lung, respectively. In our series, the linear 0.6%/y rate of development of lung MSPCs noted during the first 10 postoperative years contradicts the data reported by Engelen et al[15] in a series based on 556 patients with squamous cell carcinoma of the larynx and a median observation duration of 37 months; they noted that the incidence curve for lung MSPCs in patients with glottic carcinoma sloped down after 2 years and became flat after less than 5 years. Such a discrepancy might be explained by differences between the studies in terms of follow-up and exclusion criteria. On the other hand, the regular 1%/y rate of development of upper aerodigestive tract MSPCs and the 0.6%/y rate of development of lung MSPCs in our series are in line with the previously reported 1.3%/y rate of development of second respiratory tract tumors noted by Wagenfeld et al[5] in patients with stage I glottic carcinoma treated with radiotherapy alone. However, this rate of development was lower than the 2.5%/y rate of development of MSPCs in glottic tumors classified as T1-2 managed with radiotherapy and the 4.8%/y rate of development of MSPCs in glottic tumors classified as T3-4 managed with radiotherapy reported by Roberts et al.[8] Such a difference supports, in our opinion, the notion of a possible etiologic role for irradiation in carcinogenesis of the larynx, as already suggested in 1975 by Lawson and Som[7] in a series of 535 patients with a glottic carcinoma classified as T1 and a minimum of 5 years of follow-up. They noted that the overall incidence of MSPCs of the larynx varied from 3.9% when the conservative laryngeal treatment was partial laryngectomy to 8% when the conservative laryngeal treatment was radiotherapy. Such data, in our opinion, should lead to further studies of the possible etiologic role of radiotherapy of laryngeal cancer in the development of MSPCs.

In univariate analysis, the development of MSPCs at the time of partial laryngectomy was statistically more likely in smokers than in nonsmokers (Table 1, p = .04). This observation is consistent with the old concept of the increased susceptibility of the mucosa to carcinogenic exposure. However, no statistical correlation could be noted between the amount of tobacco intake and MSPCs (Table 1, p = .17). Furthermore, we were not able to determine any differences in incidence rates based on continued smoking after treatment versus cessation, as this information was not systematically registered in the follow-up visit notes. Interestingly, in our report, no significant statistical relationship could be demonstrated between the use of an induction chemotherapy regimen before partial laryngectomy and the development of MSPCs (Table 1, p = .44). This last finding contradicts the concept that the use of induction chemotherapy in patients with laryngeal cancer allows for "cleansing" of distant sites that helps prevent subsequent development of related cancers. The lack of a statistical relationship between the T classification of the true vocal cord carcinoma and the incidence of MSPCs in our series (Table 1, p = .95) also contradicts the data of McDonald et al,[16] who reported a higher incidence of MSPCs in early-stage laryngeal cancer (T1 and T2) as compared with all laryngeal cancers, as well as the data by Roberts et al,[8] who reported a higher incidence of MSPCs in "early"-stage glottic cancers (T1 and T2) than in "advanced"-stage glottic cancers (T3 and T4). Such a discrepancy might be explained by the retrospective and nonrandomized nature of the reports, the differences in the minimum duration of follow-up, the low number of patients with cancer classified as T3-4 (18/410) in our series, and/or the higher cure rates achieved for T1-2 laryngeal tumors as compared to other laryngeal cancers (giving therefore more "chances" for patients with T1-2 laryngeal cancer to develop MSPCs as compared to patients with T3-4 laryngeal cancers).

In our series, survival was statistically more likely to be reduced in patients who developed MSPCs than in patients who did not develop MSPCs. The 10-year actuarial survival estimate varied from 43.7% in patients who developed MSPCs to 76.8% in patients who did not develop MSPCs (p < .0001). Such findings confirm the general notion that MSPCs are increasingly responsible for disease-specific mortality as the control of primary laryngeal cancers improves.[5-8,15-19] The results reported in terms of survival in our series are also in line with the data reported by Fujita et al[6] (in a series of 158 patients with T1 glottic carcinoma and a minimum follow-up of 12 months managed with radiotherapy alone), who noted that the 10-year overall survival rate varied from 57% for patients who did not develop MSPCs to 26% for patients who developed MSPCs ($p = .003$). However, of utmost interest is the fact that the 10-year actuarial survival estimate of 43.7% noted in the group of patients who developed MSPCs in our series was far better than the 26% 10-year overall survival estimate reported by Fujita et al. Although such a comparison between the series suffers from numerous biases (the retrospective nature of both reports, the inclusion of patients with MSPCs at sites other than the upper aerodigestive tract, the lack of an actuarial method for analysis of survival in the report by Fujita et al), the suggested negative impact (in terms of long-term survival) of radiotherapy as compared to partial laryngectomy as the initial conservative treatment modality is consistent with the recent report by Dolan et al,[19] who noted that the survival of patients with MSPCs was poorest in those patients whose prior head and neck cancer had been treated with radiotherapy, because of the reduced possibility of achieving the most effective treatment of the MSPCs. In our opinion, this important notion should be kept in mind when deciding on the treatment modality (partial laryngeal surgery versus radiotherapy) to be offered to patients with true vocal cord carcinoma amenable to conservative laryngeal treatment.

In our series, the 10-year actuarial survival estimates were 24% for patients who developed lung MSPCs, 43.7% for patients who developed non-lung, non–upper aerodigestive tract MSPCs, and 63.4% for patients who developed upper aerodigestive tract MSPCs. This poor prognosis for survival in the group of patients with lung MSPCs noted in our series was in line with previous reports analyzing survival in patients who were initially managed for laryngeal carcinoma and who developed MSPCs.[5-8,15-19] Such data made us wonder whether the routine chest radiography examinations conventionally performed on a yearly basis were valuable in terms of early diagnosis of lung MSPC, or whether follow-up should not be modified toward the use of lung computed tomography scans in an attempt to increase the early detection of lung MSPCs. Unfortunately, according to the report by Tan et al,[20] the use of lung computed tomography scans in patients with advanced head and neck cancer did not add to the sensitivity of the screening for lung MSPCs, and it increased the overall cost. For this reason, and because the survival rate in patients with lung cancer detected by yearly radiography has been reported to be significantly higher than that in patients with lung cancer diagnosed after symptoms,[15] we still advocate at our department the use of routine yearly chest radiography in the follow-up of patients with laryngeal cancer.

In conclusion, the large number of patients (410) in the current study and the fact that 94.7% of the patients were followed for at least 10 years or until death allowed for a unique analysis of the consequences of MSPCs in patients with invasive squamous cell carcinoma of the true vocal cord successfully treated with partial laryngectomy. As with any of the prior reported retrospective studies devoted to the analysis of MSPCs following conservative treatment for glottic carcinoma,[5-9,21] the current report lacked the rigor of prospective study designs that detail systematic and uniform patient evaluations over time. However, numerous efforts were made to minimize the various biases inherent to the retrospective nature of our report. A minimum delay of 10 years from the initial partial laryngectomy was elicited to avoid the bias related to an insufficient duration of follow-up. The Kaplan-Meier[13] actuarial life-table method was used to evaluate the incidence and survival estimates to avoid the bias related to any overall crude analysis. As our main goal was to document the incidence, site of occurrence, risk factors, and outcome of MSPCs following the successful treatment of invasive squamous cell carcinoma of the true vocal cord with partial laryngectomy, we excluded from our review all patients who failed the initial partial laryngectomy. We were well aware that by excluding such patients we introduced a selection bias that might lead us to overestimate the rate of developing MSPCs. However, the inclusion of such patients would have also led to including a large group of patients who had total laryngectomy as salvage treatment.[21] This would have resulted in another selection bias leading to underestimating the rate of developing MSPCs within the remaining larynx and would have increased the risk of the subsequent development of distant metastases that might be extremely difficult to distinguish from MSPCs.[21] We therefore believed that by eliminating the patients who failed partial laryngectomy we created a more homogeneous group for study.

The data reported in the current series, together with the published medical literature, support the need for lifelong routine follow-up of patients cured of an initial invasive squamous cell carcinoma of the true vocal cord. Such a follow-up should be based upon regular otolaryngological examinations together with regular medical visits to an internist, since 1) more than half of the MSPCs noted in our series occurred in the lung or other non–upper aerodigestive tract sites and 2) the incidence curve of the MSPCs was linear. The other striking finding in our series was the highly negative impact of MSPCs on survival. As smoking was the only variable that statistically increased the risk of the development of MSPCs, we believe that the current campaigns against tobacco consumption should be continued. Furthermore, as recently underscored by Armstrong and Meyskens[22] in a review article, it appears that chemoprophylaxis in cured head and neck cancer patients provides an opportunity to arrest and reverse neoplastic progression. Since the pioneering work by Hong et al,[23] various reports have noted that retinoids have shown promising results in reducing the incidence of MSPCs in the head and neck region, but they also produced significant side effects.[22] Currently, several other compounds (green tea, vitamin E, nonsteroidal anti-inflammatory agents, and protease inhibitors) are under investigation,[22] and they might provide in the near future a clue to reducing the incidence of MSPCs in cured glottic carcinoma.

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ASSOCIATION OF EPSTEIN-BARR VIRUS AND NASOPHARYNGEAL CARCINOMA IN CAUCASIAN PATIENTS

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We evaluated the association of nasopharyngeal carcinoma (NPC) and Epstein-Barr virus (EBV) in Spanish patients, and studied the expression of EBV products (latent membrane protein–1 [LMP-1] and ZEBRA proteins) by NPC cells and its possible prognostic value. In situ hybridization (ISH) for EBV-encoded nonpolyadenylated RNAs (EBERs) and immunohistochemical expression of LMP-1 and ZEBRA proteins by immunohistochemistry were examined in formalin-fixed, paraffin-embedded NPC specimens from 30 patients, and a survival analysis was done by the Kaplan-Meier method. We detected EBERs by ISH in 96.67% of the NPC cases, and detected expression of LMP-1 in 43.33% of the NPC cases and expression of ZEBRA protein in 6.67% of the NPC cases. We conclude that ISH for expression of EBERs is an adequate method for detection of EBV in NPC. LMP-1 is not frequently expressed in NPC cells (43.33%). Most NPC cells carry a latent EBV infection. LMP-1 expression might have worsened the prognosis of NPC in our series.

KEY WORDS — Epstein-Barr virus, in situ hybridization, latent membrane protein–1, nasopharyngeal carcinoma, ZEBRA protein.

INTRODUCTION

Epstein-Barr virus (EBV) is a lymphotropic herpesvirus associated with several lymphoid malignancies,[1] but it has also been related to nasopharyngeal carcinoma (NPC), an epithelial neoplasm prevalent in southern China.[2] This association has been confirmed by detection of EBV antigens in NPC by immunoblotting, detection of EBV DNA by nucleic acid hybridization, detection of EBV DNA by in situ hybridization (ISH), detection of EBV genome by polymerase chain reaction (PCR), and demonstration of monoclonality of EBV in NPC.[2] In situ hybridization has been used to detect the presence of EBV-encoded nonpolyadenylated RNAs (EBERs) in neoplastic cells of NPC.[3] Further, the study of the EBV products expressed by NPC cells, such as latent membrane protein–1 (LMP-1) and the EBV lytic product ZEBRA protein (BZLF1 protein), has attracted great interest, as they may vary according to the infected cells[4] and may modify the prognosis of the disease.[5]

There is still controversy about the method of choice for detecting EBV presence in NPC specimens.[6] Both PCR for DNA and ISH for EBERs seem to be sensitive and rapid, but ISH for EBERs has the added advantage of locating the presence of EBV only in neoplastic cells.[7,8] Both methods have demonstrated the presence of EBV in NPC no matter the degree of differentiation of the tumor, although it is more frequently present and more intensely expressed in undifferentiated cases.[9-11] Most reports on the association between NPC and EBV following ISH for EBERs are based on Asian cases.[8,10,12-16] Only a few series have been published on Western cases.[7,9,11,17,18] Despite the variable incidence of the disease in different geographic areas, EBV seems to be related to NPC regardless of geographic origin, race, age, or sex.[2,10]

The expression of EBV products by NPC cells is variable,[1,2] but most series show that EBV infection in NPC cells is characterized by the expression of latent proteins such as EBV nuclear antigen–1 (EBNA-1) and LMP-1, following the so-called type II pattern of latency.[4,19] While expression of EBNA-1 is fairly constant through the different series,[2] the frequency of expression of LMP-1 varies, from 18%[20] to 78%[21] of cases. Although this variability might have epidemiological causes, it seems to be more related to technical considerations.[2,7,9,22,23]

Latent membrane protein–1 is the only EBV oncogene capable of transforming rodent fibroblasts in vitro, and it is essential for primary B-lymphocyte transformation.[1,2] Few studies have found a prognostic relationship between LMP-1 expression and NPC. Hu et al[5] reported a more rapid and expansive course

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of disease in LMP-1–positive cases, which were nevertheless associated with a better prognosis.[5]

Expression of lytic EBV products, such as ZEBRA protein, by NPC cells has not been described as a common feature, but it has been reported in some neoplastic cells in some cases.[2,13,24-26] The fact that some NPC cells express ZEBRA protein might partially explain the so-called serologic EBV lytic response in NPC patients.[27]

The purpose of this study was to evaluate whether NPC is associated with EBV in Spanish patients, and to study the expression of EBV products (LMP-1 and ZEBRA proteins) by NPC cells and any possible prognostic value.

MATERIAL AND METHODS

Tissue Samples and Histologic Classification. For this study, we selected 30 NPC cases from the archives of the Pathology Department of the Hospital Ramón y Cajal (1977 through 1996). Thirty-two formalin-fixed, paraffin-embedded NPC specimens (30 nasopharyngeal biopsies, a cervical metastatic adenopathy, and a bone marrow metastasis) were analyzed. Classification of NPC was performed on 5-µm nasopharyngeal sections stained with hematoxylin and eosin. The NPC specimens were subclassified according to the World Health Organization (WHO) classification[28] into type I (keratinizing squamous carcinoma), type II (nonkeratinizing squamous carcinoma), and type III (undifferentiated squamous carcinoma). The classification system proposed by Hsu et al[29] was also used: KS (keratinizing squamous carcinoma), RC (red cell squamous carcinoma), FS (fusiform cell squamous carcinoma), and MX (mixed cell squamous carcinoma). The Hsu degree of differentiation (A or B) was also used in RC cell type cases.[29] The clinical stage at presentation was established according to the TNM and Ho[30] systems.

In Situ Hybridization for EBERs. In situ hybridization for EBERs was performed according to a nonisotopic protocol described elsewhere.[14] All solutions and reagents were prepared with water treated with diethylpyrocarbonate. Tissue 4-µm sections from formalin-fixed, paraffin-embedded NPC specimens were deparaffinized, dehydrated, predigested with pepsin, and hybridized overnight with a fluorescein-conjugated EBV oligonucleotide probe for EBERs at 0.25 ng/µL (Dako, Glostrup, Denmark). Hybridization products were detected with a mouse monoclonal anti–fluorescein isothiocyanate (FITC; Dako) and rabbit polyclonal anti-mouse antibodies conjugated with alkaline phosphatase (Dako). Diaminobenzidine was used as a chromogen, and slides were counterstained with hematoxylin. Positive staining, while present in more than 50% of tumoral cells, was recognized microscopically as dark brown granules at the site of hybridization, usually limited to the nucleus of neoplastic cells. Hodgkin's disease in human immunodeficiency virus–positive patients was used as a positive control, and other non–EBV-related neoplasms were used as negative controls (8 colonic adenocarcinomas, 6 breast adenocarcinomas, 7 laryngeal carcinomas, and 5 neck metastatic adenopathies).

Immunohistochemistry. Immunohistochemical detection of LMP-1 and ZEBRA protein was performed on routinely processed paraffin wax sections by means of CS1-4 antibodies (Dako) and BZLF-1 antibody (Dako), respectively. A standard avidin-biotin method was used.[23] LMP-1 expression was considered positive when more than 5% of neoplastic cells showed a membrane pattern of staining, and ZEBRA expression was considered positive if nuclear staining was found in more than 5% of neoplastic cells. Abolition of the antibody was used as a negative control. Hodgkin's disease in an immunocompromised patient was used as a positive control for LMP-1 detection, and an oral hairy leukoplakia was used as a positive control for ZEBRA detection.

Statistical Analysis. Comparison of the distribution of categorical data between groups was done with the 2-tailed Fisher's exact test. Survival analysis was performed by the Kaplan-Meier method. We considered p values of $\leq .05$ to be statistically significant.

RESULTS

Histologic Classification. Age at onset, sex, histologic subtype (WHO,[28] Hsu[29] system, Hsu[29] degree of differentiation), clinical stage at presentation, survival status, and length of follow-up are summarized in Table 1.[2,28-30] Briefly, patient ages ranged from 15 to 81 years, with a median of 49.87 years (SD, 16.22 years). There were 18 male and 12 female patients (ratio, 1.33). All but 1 of the patients were white, and the other was an Asian woman. Four cases were classified as WHO type I, 2 as WHO type II, and 24 as WHO type III. According to the Hsu system, 5 cases were classified as KS cell type, 2 as FS cell type, 3 as MX cell type, and 20 as RC cell type. Eleven RC cell type cases were type A (highly undifferentiated) NPC, and 9 cases were type B. The clinical UICC[29,30] stage at presentation was available for 27 patients: 5 presented with stage II, 7 with stage III, and 15 with stage IV. All the cases with cervical regional extension had metastatic nodes staged N2 or greater by the Ho[30] staging system.

ISH for EBERs. We detected EBER expression in 29 of the 30 NPC cases evaluated (96.67%) and in the 2 metastases included in this series. The positive hybridization for EBERs was demonstrated as nu-

TABLE 1. SUMMARY OF RESULTS

No.	*Age*	*Sex*	*WHO*[28] *Type*	*Hsu*[29] *Type*	*Clinical Stage UICC*[2]	*Ho*[30] *N Stage*	*Status and Follow-up*	*ISH for EBERs*	*LMP-1 Expression*	*ZEBRA Expression*
1	55	M	III	RC A				+	–	–
2	52	F	III	RC A				+	+	–
3	57	M	III	RC B	IV	N2	NED, 12 y	+	–	+
4	50	M	III	RC B	IV	N2	DOD, 1 y	+	–	–
5	44	M	III	RC B				+	–	–
6	15	M	III	RC A	IV	N3	DOD, 1 mo	+	+	–
7	74	F	III	MX	IV	N2	DOD, 4 mo	+	–	–
8	47	M	III	MX	III	N2	DOD, 16 mo	+	+	–
9	57	M	III	RC B	IV	N3	DOD, 6 mo	+	–	–
10	71	F	III	RC A	IV	N2	DOD, 17 mo	+	+	+
			III	RC A				+	+	–
11	67	F	III	RC A	III	N2	DOD, 5 y	+	–	–
12	20	M	III	RC B	IV	N2	DED, 3 y	+	–	–
			III	RC B				+	+	–
13	55	M	I	KS	IV	N2	DOD, 18 mo	+	–	–
14	56	M	III	FS	IV	N2	NED, 9 y	+	–	–
15	47	M	III	FS	II	N0	DOD, 20 mo	+	–	–
16	48	M	II	KS	II	N0	DOD, 5 y	+	–	–
17	22	M	III	RC B	III	N2	NED, 9 y	+	+	–
18	28	M	III	RC B	IV	N2	NED, 7 y	+	+	–
19	81	M	III	RC A	IV	N0	DOD, 18 mo	+	+	–
20	44	F	III	RC A	IV	N2	DOD, 3 y	+	–	–
21	42	F	II	MX	IV	N2	NED, 7 y	+	+	–
22	32	F	III	RC B	III	N2	DOD, 18 mo	+	+	–
23	64	F	I	KS	IV	N2	DOD, 9 mo	+	–	–
24	47	F	I	KS	III	N2	NED, 5 y	+	–	–
25	56	M	III	RC B	II	N0	NED, 5 y	+	–	–
26	72	M	I	KS	II	N0	DOD, 18 mo	–	–	–
27	28	F	III	RC B	IV	N3	DOD, 15 mo	+	+	–
28	52	M	III	RC B	II	N0	NED, 3 y	+	–	–
29	57	F	III	RC A	III	N0	NED, 3 y	+	+	–
30	56	M	III	RC A	III	N0	DOD, 7 y	+	+	–

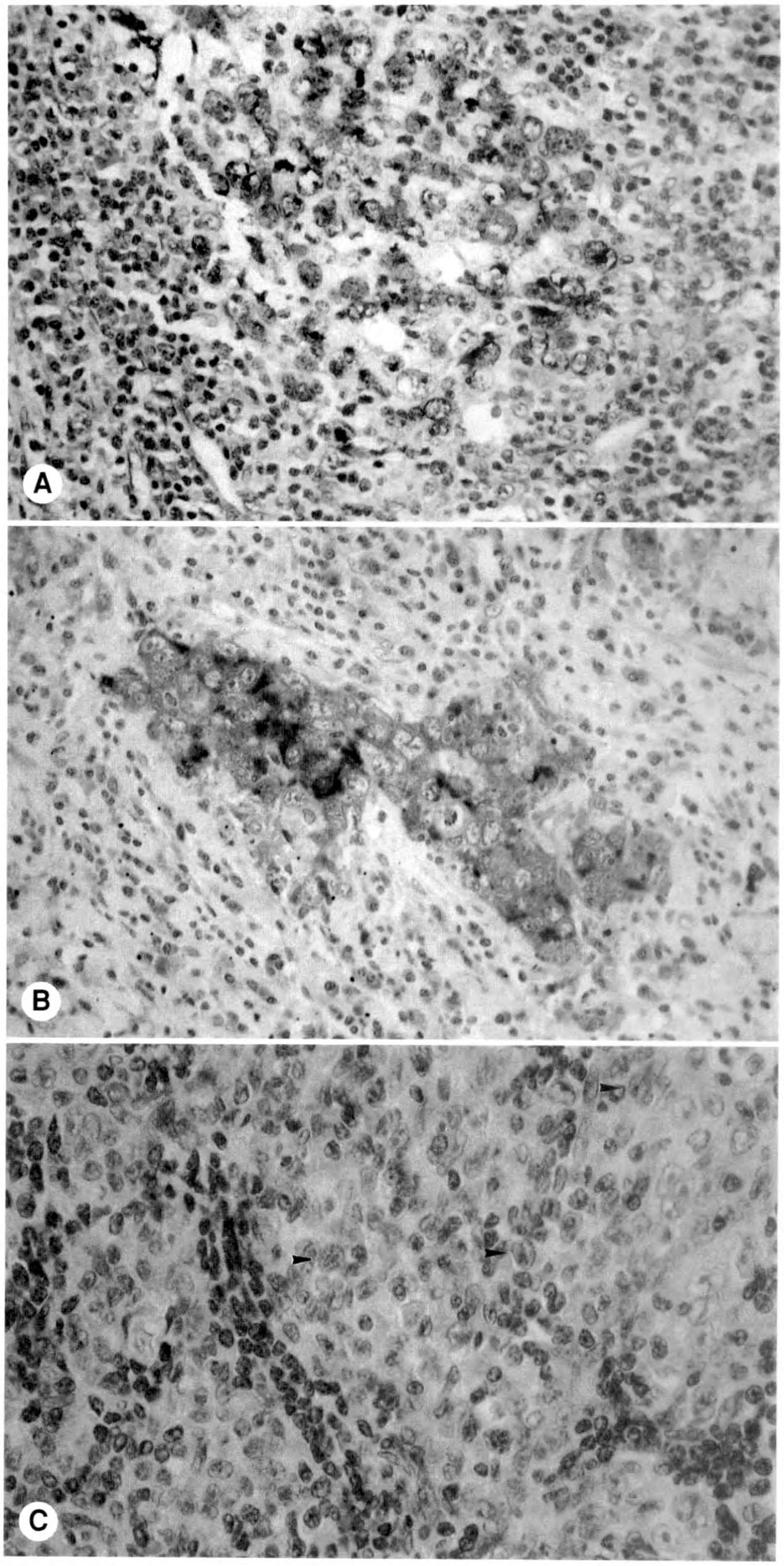
Summary of clinical, histologic, molecular (ISH for EBERs), and immunohistochemical results for series of 30 NPC cases (32 specimens, including 30 nasopharyngeal biopsies, 1 cervical metastatic node, and 1 bone marrow metastasis). Note that case 10 has second specimen (node metastasis) and that case 12 also has second specimen (bone marrow metastasis).

NED — no evidence of disease, DOD — dead of disease. See text for other abbreviations.

clear staining that involved most of the neoplastic cells in most specimens, whereas surrounding reactive lymphoid tissue showed no staining (see Figure, A). The only case negative for EBV was a WHO type I NPC (Table 2).

Immunohistochemistry. Expression of LMP-1 was observed in 13 of the 30 NPC cases (43.33%), whereas expression of ZEBRA protein was observed only in 2 of the 30 NPC cases (6.67%). In the LMP-1–positive cases, only isolated NPC cells showed membrane expression, and characteristically, the intensity of the staining was weak in most cases. LMP-1 expression was restricted to scattered cells in several areas of the specimens (see Figure, B). Plasma cell staining was considered cross-reactivity of LMP-1. ZEBRA protein was expressed in only 2 cases, showing its characteristic nuclear pattern, and only in some neoplastic cells (see Figure, C). Both EBV proteins were only expressed by tumoral cells. LMP-1 and ZEBRA protein expression could not be correlated to tumor histology, because most cases of the present series were WHO type III. Nevertheless, the 4 WHO type I cases in this series were LMP-1–negative, and only 1 of the 2 WHO type II cases was LMP-1–positive (Table 2).

Impact of EBV Product Expression on NPC Survival. A survival analysis of the whole series was available for 27 patients and showed a 5-year survival rate of 32.77% (95% confidence interval [CI], 14.06% to 52.09%) after a mean follow-up of 62.74



Nasopharyngeal carcinoma, World Health Organization type III (original ×40). **A**) Positive in situ hybridization for Epstein-Barr virus–encoded nonpolyadenylated RNAs, exclusively located in tumoral cells. **B**) Expression of latent membrane protein–1 in most of tumoral cells. **C**) Weak positive expression of ZEBRA (BZLF1) protein, restricted to some scarce tumoral cells (arrowheads).

months. The most significant prognostic clinical factor was regional extension. Thus, the presence of neck metastatic nodes reduced the 5-year survival rate from 50% to 26.64% (p = .003). Survival analysis in relation to WHO type could not be done, as most cases were type III.

As nearly all the cases were related to EBV, it was not possible to evaluate the prognostic significance of this association. Nevertheless, LMP-1 expression,

TABLE 2. COMPARISON OF METHODS

Histology	*ISH for EBERs*	*LMP-1 Expression*
WHO type I	3/4 (75%)	0/4 (0%)
WHO type II	2/2 (100%)	1/2 (50%)
WHO type III	24/24 (100%)	12/24 (50%)
	29/30 (96.67%)	13/30 (43.33%)

Comparison between EBV DNA ISH for EBERs and LMP-1 immunohistochemical expression in detection of EBV association with NPC. Data are positive cases/total cases. See text for abbreviations.

only present in 43% of the cases, was associated with a worse prognosis (0% survival rate at 5-year follow-up in LMP-1–positive cases, compared to 30.33% in LMP-1–negative cases), although this association was not statistically significant (p = .065).

DISCUSSION

Molecular EBV studies have demonstrated that NPC is uniformly related to EBV[2] and that EBV remains in infected NPC cells in a latent phase of infection, following a type II pattern of latency.[19] Initial reports stated that EBV was only related to undifferentiated (WHO type III) NPC,[9,11] but more recent reports have shown that all NPCs are associated with EBV, irrespective of WHO type.[7,10,12,13,15]

Our series has shown that almost all Spanish NPC cases are EBV-infected and that ISH for EBERs is the best method for the detection of EBV in NPC. It is a very sensitive method (96.67%) and is able to locate EBV infection restricted to neoplastic cells.[9] Thus, in agreement with Pathmanathan et al[10] and Murono et al,[12] we consider ISH for EBERs to be the ideal technique for detection of EBV infection in NPC specimens. In situ hybridization for EBERs has been reported to demonstrate EBV infection in 32% to 100% of various series.[7-18] Most authors have observed that EBER expression is more intense in undifferentiated cases[31] and that it correlates well with EBV PCR findings.[6,7,10] Few reports are based on Western patients, but Gulley et al[9] observed 71% of NPC cases expressing EBERs by ISH out of a series of 45 patients from the United States and Canada, and Vera-Sempere et al[7] found in 76% of NPC Spanish cases positive nuclear signals for EBERs by ISH.

The fact that LMP-1, a latent membrane protein expressed by EBV-infected cells, is one of the EBV proteins with demonstrated oncogenic properties makes the detection of LMP-1 expression by NPC cells very interesting.[2] While EBNA-1 is uniformly expressed by neoplastic infected cells, LMP-1 expression is very variable,[2,5,9,10,13,18,20,21,32-36] ranging from 18%[20] to 78%[21] of cases. This variability might be related to epidemiological factors (different EBV strains in diverse geographic areas),[9] to the degree of differentiation of the tumor (usually more intense in differentiated cases),[24] and/or to methodological variations such as fixation difficulties, monoclonal antibodies used, or the fact that studies based on immunoblot or reverse transcription–PCR are more sensitive than those based on immunohistochemistry.[22] Our results (43.33% LMP-1–positive NPCs) confirmed that LMP-1 expression is not a good screening method for detecting EBV associated with NPC specimens, while EBER expression by ISH is an accurate method for this purpose.[9,10,13] In addition, LMP-1 expression was usually weak and restricted to some isolated NPC cells, while EBER expression was more intense and was present in most of the neoplastic cells.[9]

Further, 56.66% of NPC cases with abundant EBERs did not express LMP-1. This finding shows that more than half of NPC cases do not follow the so-called type II pattern of latent EBV infection, because of a lack of expression of LMP-1. Further, this finding supports the notion that LMP-1 may not be necessary for developing or maintaining NPC.[9] Instead, LMP-1 might play a significant role in an earlier stage of tumor development[10,37] and be down-regulated thereafter, probably through clonal selection of LMP-1–negative tumor cells by immunologic pressure,[36] because EBV-specific cytotoxic T-cells are potentially directed against latent proteins other than the often-unrecognized EBNA-1, such as LMP-1.[38]

Expression of lytic EBV products, such as ZEBRA protein (BZLF1 protein), by NPC cells has not been described as a common feature, but it has been reported in some neoplastic cells in some cases.[24-26] Gulley et al[39] reported ZEBRA protein expression in a few cases (4 of 42 cases; 10%), and it was not colocalized with p53 accumulation. The fact that few NPC cells express ZEBRA protein does not fully explain the so-called serologic EBV lytic response in NPC patients.[27] Some authors[26,40] have claimed NPC cell expression of the lytic (truncated) form of LMP-1, explaining the co-expression of ZEBRA protein. Others[37,39] have shown that ZEBRA expression is detected only in rare cells, whereas LMP-1 and EBERs are observed in most cells. Our series has also shown some NPC cases with ZEBRA protein expression in some neoplastic cells, confirming its possible role in the pathogenesis of the disease. Thus, this infrequent ZEBRA expression in NPC confirms that EBV infection in these tumors is in a latent state.[37]

Finally, EBV infection was examined with regard to its effect on the prognosis of NPC. We observed that LMP-1–positive NPC cases showed a worse prognosis, and there were no LMP-1–positive NPC survivors after the 5-year follow-up, although this difference was not statistically significant, possibly because of the small number of cases. LMP-1–positive NPC cases have been reported as tumors with a more rapid and expansive growth, nevertheless associated with a better prognosis, while negative cases had a greater tendency to recur or metastasize.[13] The series by Hu et al[5] included 74 patients from Shanghai, and 66% were LMP-1–positive by immunoblotting. The difference between their series and ours might be that they used a more sensitive method to evaluate LMP-1 expression and that their cases were not assessed by survival; instead, LMP-1 expression was only compared to clinical stage and recurrence.

Therefore, according to these results, NPC in Spanish patients is related to EBV in almost all cases, irrespective of their histologic grade.[7,10] This relationship is best assessed by ISH for EBERs because of its sensitivity and ability to locate EBV infection in neoplastic cells. Thus, ISH for EBERs has become a useful adjuvant in the early diagnosis of NPC[41] and in the management of recurrent NPC.[42] However, LMP-1 expression in NPC is found in less than half the cases, and EBV lytic infection in NPC is rare. LMP-1–positive NPC cases have a tendency to have a worse prognosis than LMP-1–negative ones. Further investigation is warranted to fully understand EBV interaction with NPC.

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MEDIAN CLEFT OF THE LOWER LIP: REPORT OF TWO NEW CASES AND REVIEW OF THE LITERATURE

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Median clefts of the lower lip and mandible are rare craniofacial clefts. Couronné in 1819 was the first to describe the condition. The midline cleft of the lower lip was classified by Tessier as a type 30 craniofacial cleft. Recently, the total number of the reported cases had increased to about 66 in the world literature. In addition, 2 more patients are presented here. The first case involves only a small notch in the vermilion; the deformity was treated by Z-plasty. In the second case, a midline incomplete cleft of the lower lip, a sublingual abnormal frenulum, a complete cleft in the mandible, a bifid sternum, a presternal skin tag, and a ventricular septal defect in the heart were present. At operation, Z-plasty of the lingual frenulum released the normal-sized tongue. The lip cleft was corrected by a simple V excision followed by closure in 3 layers. The mandibular segments were "vitalized" with a bone rongeur and immobilized in the predetermined position with an interosseous stainless steel wire. Because the bone fusion was complete, the stainless steel wire was taken out after 3 months so that it would not prevent mandibular development. Our treatment methods and others are discussed.

KEY WORDS — lower lip cleft, midline cleft, Tessier No. 30 cleft.

INTRODUCTION

Median clefts of the lower lip and mandible are rare craniofacial defects. Couronné in 1819 was the first to describe the condition.[1] The midline cleft of the lower lip was classified by Tessier[2] as a type 30 craniofacial cleft. Fujino et al[3] listed 34 cases of a cleft of the lower lip that had been reported in the literature and added 5 more cases of their own. By 1984, the number of published cases had increased to 58.[4] More recently, the total number of reported cases had increased to about 66 in the world literature.[5] It is probable that some cases have gone unreported or been missed.

Median mandibular clefts vary in their presentation, ranging from minor clefting of the lower lip[6] to complete clefts of the mandible with absence of the hyoid bone, thyroid cartilage, and manubrium.[7,8] Most of the previously reported cases showed only a median cleft of the lower lip and mandible, with the remainder having various associated anomalies, including a cleft of the tongue, ankyloglossia, oligodontia, heart anomalies, and malformation of the sternum and extremities. Possible associated anomalies are congenital heart deformities,[1,8-12] cleft palate,[1,3,4,10,13] facial anomalies (such as submental epidermoid cysts,[14,15] hemifacial microsomia,[16] Pierre Robin anomaly,[4] and eye and ear deformities[3,8,10,12]), hand anomalies (such as syndactyly, brachydactyly, and polydactyly),[1,17] foot deformities (such as clubfoot and ectrodactyly),[10,17] and chromosomal aberrations.[1,18]

From an etiologic point of view, a median cleft of the lower lip is thought to be a failure of the fusion of the lower part of the first branchial arch, resulting in not only a cleft of the lower lip, but also a cleft of other structures coming in from the lower part of the first branchial arch.[1,19] Oostrom et al[20] tried to explain the causation of the median cleft of the lower lip and mandible from an embryological point of view. According to the authors, instead of paired branchial arches, only 1 first branchial arch develops during the early embryonic period. Within this first branchial arch, 2 mandibular processes grow outward, separated by a median groove. These mandibular processes do not fuse, but merge during the late embryonic period (crown-rump length, 17 to 60 mm). Hypoplasia of the mandibular processes during the early embryonic period will lead to severe clefts of the lower lip and mandible, and defects in the merging process in the late period will produce higher clefts of the lower lip.[20]

The rarity and variation in severity of this condition are responsible for the lack of consensus on the mode of management and the timing of surgical procedures.[7,8] The purpose of this article is to report 2 new cases of median cleft of the lower lip and to discuss the treatment methods used by us and others.

CASE 1

A 17-year-old girl was admitted to the Plastic and Reconstructive Surgery Service of the Ankara Numune Hospital (Turkey) with a congenital deformity of the lower lip (Fig 1A). The alveolar process and the basis of the mandible were intact. The deformity

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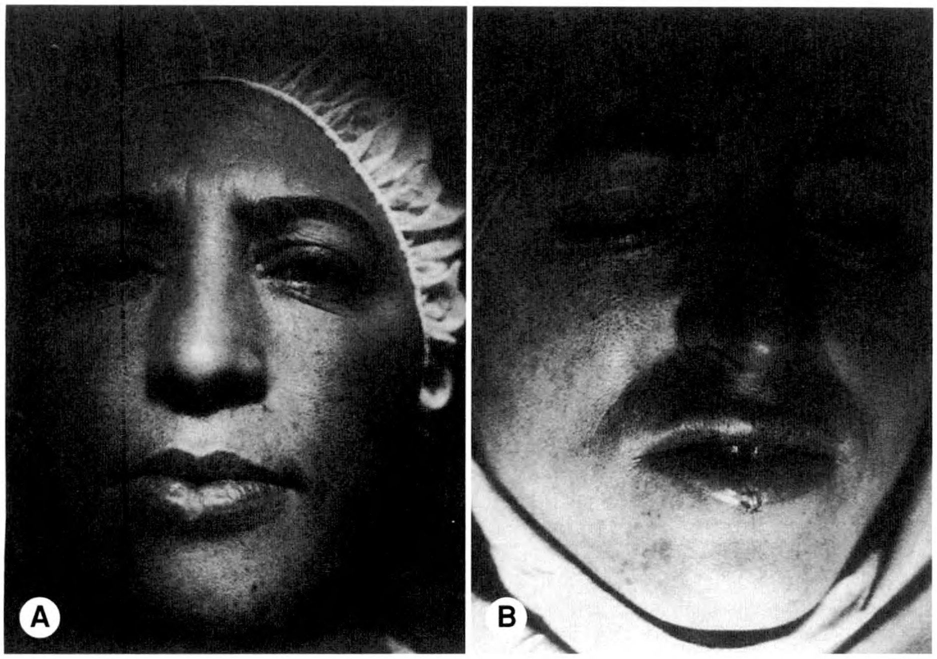

Fig 1. (Case 1) **A)** Preoperative view. **B)** Postoperative view.

was treated with a Z-plasty (Fig 1B).

CASE 2

A 10-month-old male infant had been born after a normal gestation of 38 weeks. He weighed 3,300 g and was a second-born child. The pregnancy had been uneventful. The mother had no known exposure to medication or radiation. There was no history of consanguineous marriage nor any record of congenital deformity in the family. The mother was 24 years of age and the father 21 years; both were in good health.

The child showed the following anomalies, none of which were life-threatening.

1. There was an incomplete median lower lip cleft. The cleft was approximately one third of the lower lip in width and about 15 mm in vertical length (Fig 2A).

2. Intraoral palpation revealed that there was a midline mandibular notch and that the two halves of the mandible were freely mobile. Three-dimensional computed tomography confirmed a mandibular separation of approximately 5 mm at the symphysis (Fig 3A). The infant was breathing well and could be fed without difficulty.

3. On computed tomography of the thorax, a subcutaneous solid lesion was seen at the manubrium with a diameter of 19 mm and the appearance of a bifid corpus sterni.

4. There was a tight frenulum tethering the tongue to the groove in the alveolus. There was a minimal sulcus on the tip of the tongue, and a band like a frenulum restricting the tongue movements reached from the lower lip mucosa to the tip of the tongue (Fig 3B).

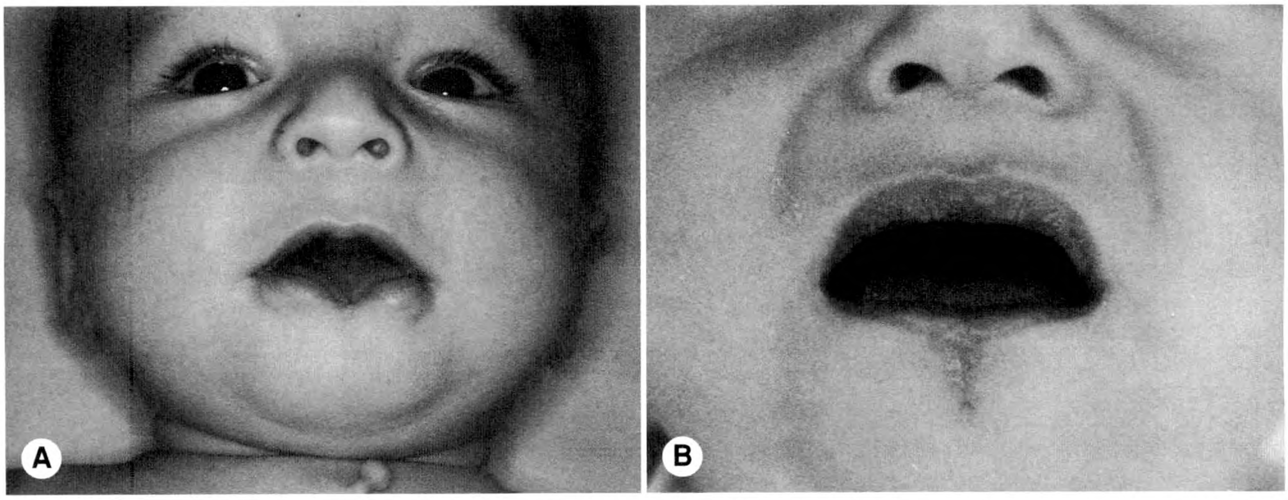

Fig 2. (Case 2) **A)** Preoperative view. **B)** Postoperative view.

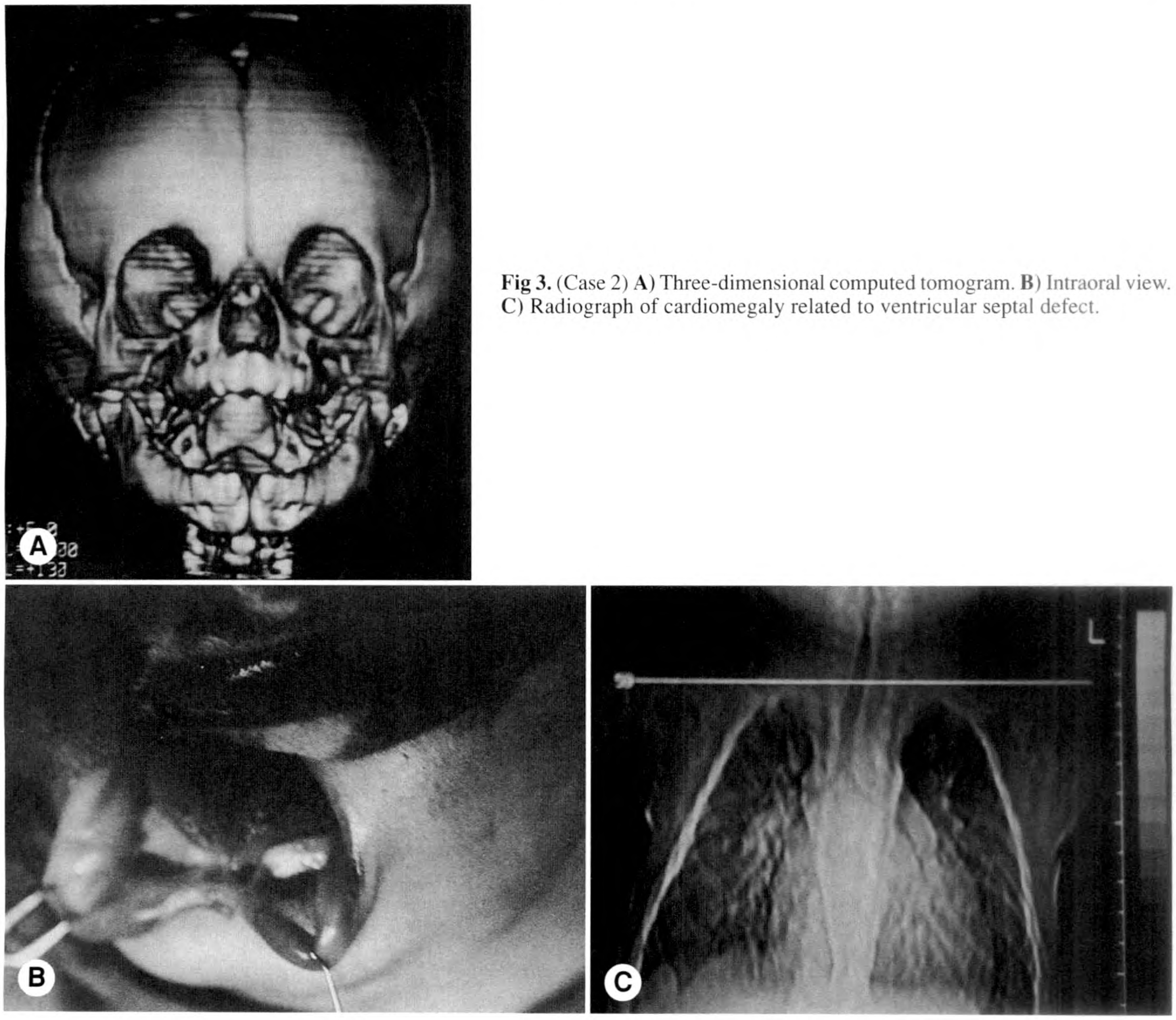

Fig 3. (Case 2) **A**) Three-dimensional computed tomogram. **B**) Intraoral view. **C**) Radiograph of cardiomegaly related to ventricular septal defect.

5. On echocardiographic examination, there was a perimembranous ventricular septal defect, increasing pulmonary current, and minimal mitral valve insufficiency. Cardiomegaly related to the ventricular septal defect was present, but the left ventricle function was normal (Fig 3C).

At operation, first, Z-plasty of the lingual frenulum was used to release the normal-sized tongue. The lip cleft was corrected by a simple V excision, followed by closure in 3 layers. The mandibular segments were "vitalized" with a bone rongeur and immobilized in the predetermined position with an interosseous stainless steel wire. Approximately 3 months after the first operation, the bone fusion was complete, and the stainless steel wire was taken out so that it would not affect the mandibular development (Fig 2B).

DISCUSSION

Median clefts of the lower lip and mandible are rare craniofacial clefts. Couronné in 1819 was the first to describe the condition.[1] The total number of reported cases in the world literature is now about 66.[5] In addition, 2 more patients are presented here.

The rarity and variation in severity of this condition are responsible for the lack of consensus on the mode of management and the timing of surgical procedures. Minor incomplete clefting of the structures of the lower lip has usually been repaired with a V excision of the cleft with a direct closure and the use of a Z-plasty if the cleft extends further down the neck.[1] The lip cleft repair and ankyloglossia release are usually performed as soon as is practicable, so that the function of the tongue and lip for swallowing, sucking, and speech is restored as soon as possible. The discussion about the timing of treatment of the mandibular defect is not closed yet. In 1879, Lannelongue reported wiring the mandible at $2^1/_2$ years of age,[1] and Wölfler,[21] Davis,[8] and Torres et al[22] performed wiring between 3 weeks and 4 years of age.

Russell and Staley[23] reported the case of a 15-year-old boy who had a soft tissue repair in infancy. Good bony union was achieved after the freshened bone ends had been fixed with an interosseous wire. Three cases were described by Petit and Psaume.[24] Although soft tissue repairs had been carried out, the children in their cases, 7 years old, were being kept under observation. At the time of the report, they had not suffered any functional disorder due to the mobility, and the question of fixation was deferred. Rather early attempts to achieve union were favored by Monroe.[1] Millard et al[25,26] reported initial correction of a midline cleft of the lower lip at 6 months, followed by closure of the mandibular cleft at 3 years. Sherman and Goulian[9] reported single-stage repair of the lip, mandible, and tongue with release of ankyloglossia at the age of 20 months with good aesthetic and functional results. Knowles et al[27] and Rea[28] reported similar cases in which bony repair was deferred to adulthood. Clefts presenting with separation of the halves of the mandible are usually corrected as a 2-stage procedure involving bone grafting of the mandibular defect.[25] Treatment of mandibular clefts is performed by most surgeons when the child is about 10 years of age to avoid damaging developing tooth buds. Our second case had relatively early surgery (10 months of age) of both the lip and mandible. We think that careful osteosynthesis of the lower border of the mandible cannot harm the tooth buds in the early period.[20] Again, this timing also depends on the severity of the clefting deformity. The cleft may be so wide that it is impossible to bridge the defect of the mandible at an early age. In our case, the mandibular cleft was about 5 mm. Being narrow, the cleft could easily be brought together, and osteosynthesis could be done with a wire at an early age. In plate screw stabilization, we believe that the roots of the teeth will be damaged. After stabilization with a wire has been made, the wire should be taken out so that mandibular development will not be affected in a negative way. In wide defects, if the patient has feeding or breathing problems, the bone segments should be stabilized at an early age with a bone graft or a reconstruction plate. If there is no such problem, the soft tissue cleft and its attachments should be corrected and the mandible defect should be closed with a bone graft around the age of 10 years. Our second patient's dental occlusion and tooth condition will be kept under observation by orthodontists in his later life.

Only 7 of the 66 patients had a congenital heart anomaly.[1,8-12,20] In our case, cardiomegaly from a ventricular septal defect was present, but because the left ventricle function was normal, the patient was given digitalis and kept under observation.

From an etiologic point of view, if the hypothesis proposed by Oostrom et al[20] is correct, in the first case the median cleft of the lower lip developed during the late embryonic period (after 7 weeks in utero), and in the second case it developed during the early embryonic period (before 7 weeks in utero).

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BIOLOGY, PREVENTION, AND TREATMENT OF HEAD AND NECK CANCER

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TOPICAL ESTROGENS COMBINED WITH ARGON PLASMA COAGULATION IN THE MANAGEMENT OF EPISTAXIS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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The aim of this study was to assess the value of topically applied estrogens in patients with hereditary hemorrhagic telangiectasia. Twenty-six patients with this disorder were treated with argon plasma coagulation and randomized into 2 groups: group A, which had postoperative application of estriol ointment ($n = 14$), and group B, which had postoperative application of dexpanthenol ointment ($n = 12$). Over a period of 12 months, the frequency and intensity of bleeding, the patient's satisfaction, and the success of the treatment were evaluated with a questionnaire. Before the operation, more than 90% of the patients in both groups complained of daily episodes of epistaxis. Twelve months after treatment, the frequency and intensity of bleeding had significantly decreased in group A as compared to group B. Of the patients in group A, 93% were satisfied with the treatment. Of the patients in group B, only 42% were satisfied with the treatment. In both groups, more than 90% of the patients were willing to undergo the same treatment again. The combined treatment approach with argon plasma coagulation and topical estriol enables us to significantly prolong the hemorrhage-free interval.

KEY WORDS — argon plasma coagulation, epistaxis, hereditary hemorrhagic telangiectasia, topical estrogen.

INTRODUCTION

Hereditary hemorrhagic telangiectasia (HHT), also known as Osler-Weber-Rendu disease, is an autosomal dominant fibrovascular dysplasia. The clinical diagnosis is based on the classic triad of recurrent epistaxis, a positive family history, and multiple telangiectasias.[1-5]

Recurrent epistaxis is by far the most common manifestation of HHT, affecting more than 90% of the patients.[6] Although there has been therapeutic and diagnostic progress over the past few years, a cure for this often burdensome disease is still not available. Different therapies have been described, including electrocauterization, hormone therapy, microembolization, septodermoplasty, and laser coagulation. However, some methods have serious side effects and complications that need to be taken into consideration.[4,7,8]

Nowadays, a widely accepted and applied treatment for recurrent epistaxis in HHT is selective coagulation of telangiectasias by various types of lasers. The aim of this local treatment is to avoid trauma to the healthy mucosa and to reduce the intensity and frequency of bleeding. We have introduced argon plasma coagulation (APC) in the treatment of patients with known HHT and telangiectasias of the nasal mucosa as a successful treatment method.[1-4] It offers effective hemostasis and has a limited penetration depth.[9,10] Results of APC treatment have already been published in previous studies.[4,5,10,11]

For further reduction of bleeding recurrences, adjuvant therapies are required. The beneficial effects of systemic application of estrogens in patients with HHT were described by Koch et al[12] in 1952. Estrogens are known to reestablish the epithelium in the mucosa by inducing a metaplasia of the nasal mucosa to thick layers of keratinizing squamous epithelium. Consequently, nasal lesions become covered so that they are protected against local trauma and the vulnerability of the mucosa is decreased.[13] However, this treatment has been controversial, because of the various side effects of systemic hormone therapy.[13-15] In order to minimize the risk of systemic side effects, we, for the first time, applied local estrogen therapy in the nasal mucosa.

The aim of the present study was to assess the value of adjuvantly applied topical estrogens after APC treatment of telangiectasias in patients with HHT.

MATERIAL AND METHODS

Study Design. In a prospective randomized clinical trial, 26 consecutive patients with diagnosed HHT

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TABLE 1. GRADING OF FREQUENCY AND INTENSITY OF NASAL BLEEDING

Frequency of Bleeding	*Intensity of Bleeding*
Grade 1 — less than once per week	Grade I — slight stains on handkerchief
Grade 2 — several times per week	Grade II — soaked handkerchief
Grade 3 — more than once per day	Grade III — bowl or similar utensil necessary

were treated for recurrent epistaxis from June 1998 to September 1999 at the Department of Otolaryngology, University Hospital Mannheim. The purpose of this study was to evaluate the efficacy of adjuvantly applied topical estrogens after APC treatment of telangiectasias. As a primary treatment, all patients underwent APC of telangiectasias of the nasal mucosa. The patients were randomized into 2 groups. Group A had APC and postoperative intranasal application of 0.5 mg of estriol ointment (ointment containing 0.1% estriol) twice a day (morning and evening; n = 14). Group B had APC and postoperative intranasal application of dexpanthenol ointment (ointment containing 5% dexpanthenol) twice a day (n = 12). The application of ointments was not "blinded," as neither the patients nor the treating physicians had any concrete expectations of the treatment advantage of the applied ointment. All procedures were performed by the first author. The main evaluation criteria were 1) the frequency of nasal bleeding, 2) the intensity of nasal bleeding, 3) the hemorrhage-free time interval, and 4) the patient's satisfaction with the treatment result.

Before and after operation, blood samples were drawn from each patient in group A for estriol serum level measurements by an enzyme-linked immunosorbent assay.

The study was approved by the ethics committee of University Hospital Mannheim, and written consent was obtained from all subjects.

Patients. Twenty-six patients (11 male, 15 female) with known HHT, recurrent epistaxis, and telangiectasias of the nasal mucosa were enrolled in this study. Group A comprised 14 patients (4 male, 10 female), and group B comprised 12 patients (7 male, 5 female). The mean age of all patients was 56 years (range, 7 to 78 years). Epistaxis was not matched for age because of the small number of cases in each subgroup. All patients had a positive family history of HHT and had undergone several therapeutic attempts to control their nasal bleeding. Nasal packing and electrocauterization had been performed in 19 patients, laser coagulation had been performed in 8 patients, and 4 patients had undergone septodermoplasty. The patients who had undergone septodermoplasty were not excluded from our study because the telangiectasia and sources of epistaxis in these patients were not only from the septum, in which the dermoplasty had been performed, but also from the turbinates, which were subject to our treatment. Because of low hemoglobin levels and severe epistaxis, 8 patients had already required blood transfusions. Five of these patients were enrolled in group A, and 3 were enrolled in group B. Patients with known coagulation disorders, eg, associated with von Willebrand's disease, factor VIII deficiencies, or platelet aggregation disorders, and patients who required anticoagulation treatment were excluded from the study.

Clinical Evaluation. The study protocol was as follows: 1) preoperative assessment by a questionnaire (questions 1 and 2); 2) randomization; 3) APC treatment of intranasal telangiectasias of all patients; 4) for group A, APC and postoperative intranasal application of 0.5 mg of estriol ointment twice a day, and for group B, APC and postoperative intranasal application of dexpanthenol ointment twice a day; and 5) assessment by a questionnaire 4 weeks and 3 and 12 months after the operation (questions 1 through 5).

On the questionnaire, the patients were asked to compare their results with their preoperative state and to answer the following questions: 1) how often do you have nosebleeds? (use a scale of grade 1 to grade 3; the grades are shown in Table 1); 2) what is the intensity of your nasal bleeding? (use a scale of grade I to grade III; the grades are shown in Table 1); 3) how long was the interval after the operation before epistaxis started to recur more often and with more blood loss? (time interval given in weeks); 4) are you satisfied with the results of the treatment? (use a scale from 1 = very satisfied to 4 = very unsatisfied); and 5) would you agree to undergo the same operation again? (yes/no).

Statistics. The statistical evaluation of the data was performed with the software package SPSS for Windows. In every statistical analysis, significance was determined at a p value of less than .05. For statistical analysis, we used the McNemar test and the Wilcoxon test.

Primary Treatment With APC. Argon plasma coagulation is based on high-frequency monopolar surgery in which one electrode is located in a tubelike applicator. Argon gas conducted through this tube is ionized at the tip of the applicator because of the electrical field and becomes electrically conductive (light-blue luminescent plasma beam). Electrical energy can be applied in a noncontact mode to the tissue surface, on which dessication and coagulation can be observed.

Our equipment consists of a generator for high-

TABLE 2. PREOPERATIVE GRADING OF NASAL BLEEDING

	Frequency of Bleeding			*Intensity of Bleeding*		
No. of Patients	*Grade 1*	*Grade 2*	*Grade 3*	*Grade I*	*Grade II*	*Grade III*
Group A (n = 14)	0	0	14	1	0	13
Group B (n = 12)	0	1	11	0	2	10

frequency current (ICC 350) and an automatically regulated argon gas supply unit (APC 300), both from ERBE Elektromedizin (Tübingen, Germany). For nasal application, we used a rigid, resterilizable applicator with a ceramic tip and a lateral opening for the gas flow at the distal end of the tip. The generator was set at 40 to 60 W, depending on the desired intensity of the effect. The argon gas flow was set at 1.2 L/min.

The nasal mucosa was anesthesized with a gauze impregnated with lidocaine 4% and xylometazoline 0.1% and then submitted to infiltration anesthesia with lidocaine 2% containing epinephrine in a ratio of 1:200,000. The telangiectasias were treated with APC in a noncontact mode until desiccation was seen. Macroscopically, the normally reddish-appearing mucosa becomes a yellow-brown color as a sign of sufficient coagulation when water loss has occurred. In cases of bleeding, the argon plasma beam was directed tangential to the mucosa surface in order to blow away the blood and expose the bleeding vessel. The increase of argon gas flow from 1.2 L/min to 2.4 L/min in these situations enabled the desired effect. To prevent the plasma beam from reaching the surrounding tissue, a silicone protection shield was applied to the APC applicator tip. A normal speculum was used for opening of the nasal vestibulum. A forehead reflector was sufficient for illumination of the nasal cavity. In selected cases, we applied a rigid 30° endoscope for better visualization of the posterior areas of the nasal cavity.

Adjuvant Treatment With Ointment. In both groups, the patients were asked to apply approximately 0.5 mL of ointment corresponding to 0.5 mg of estriol or 25 mg of dexpanthenol immediately after APC treatment twice a day into the nasal cavity. This schedule was chosen in order to maintain a sufficient estriol serum level, which is known to decrease after 8 hours according to the pharmacokinetics and pharmacodynamics of estriol in serum blood levels.[16] The patients were provided with ointment tubes with an applicator tip for the nose and instructed how much ointment to use in order to get approximately the ideal amount of 0.5 mL of ointment into the nasal cavity. In none of the patients was epistaxis associated with the application of the ointment. Three days after surgery, the nasal cavity was reviewed in our outpatient clinic. Cleaning of the nasal cavity was avoided; crusts and layers of fibrin were left attached to the mucosa.

RESULTS

The preoperative frequencies and intensities of nasal bleeding were comparable in both groups of patients. The grading of the frequency and intensity of nasal bleeding is given in Table 2. In group A, as well as group B, more than 90% of the patients claimed to have daily episodes of recurrent epistaxis. The bleeding intensity was equal to grade III in 93% of the patients in group A and 83% of the patients in group B. Before operation, no significant differences were seen between the groups in the frequency and intensity of bleeding.

No epistaxis occurred during APC treatment. Only when larger telangiectasias of more than 3 mm in diameter were coagulated with the APC beam was an immediate rupture with subsequent bleeding observed, but it could be controlled with APC. No nasal packing was required, and no additional electrocautery was necessary to control epistaxis.

In groups A and B, the results in terms of bleeding frequency and intensity observed 4 weeks after the operation were similar to those observed 3 months after the operation. In group A, 10 patients (71%) experienced nasal bleeding once or less than once a week, and 4 patients (29%) twice a week, and none of the patients claimed to have daily episodes of nose bleeding. In group B, nasal bleeding once or less than once a week was claimed by 8 patients (67%), a few times a week by 3 patients (25%), and more than once a day by 1 patient (8%).

In group A, the results observed 12 months after surgery were comparable to those observed 4 weeks and 3 months after surgery. As compared to the preoperative findings, the frequency of bleeding was significantly reduced at 12 months after surgery ($p = .003$). In group B, the frequency of bleeding 12 months after surgery had significantly improved as compared to the preoperative findings ($p = .005$), but it had worsened as compared to the status 4 weeks and 3 months after surgery. Nasal bleeding was seen less than once a week in 3 patients (25%), a few times a week in 7 patients (58%), and more than once a day in 2 patients (17%). The development of the bleeding frequency over a time period of 12 months after surgery in both groups is demonstrated in Fig 1, which refers to the 4-week assessment as a baseline for comparison.

The bleeding intensity in group A, 4 weeks and 3

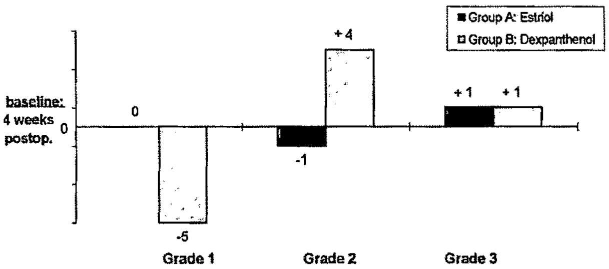

Fig 1. Frequency of bleeding 12 months after surgery, referring to bleeding 4 weeks after surgery as baseline. Indicated is number of patients whose grade of bleeding changed after 12-month assessment. Negative and positive numbers stand for loss or addition of patients in certain grade category of bleeding frequency.

months after surgery, was reduced to grade I in 10 patients (71%) and grade II in 4 patients (29%). None of the patients had grade III bleeding. Similar results were seen 12 months after surgery. Nine patients (64%) had epistaxis of grade I, and 5 patients (36%) of grade II. As compared to the preoperative findings, the bleeding intensity was significantly reduced (p = .005) 12 months after surgery. In group B, 4 weeks and 3 months after surgery, the bleeding intensity had decreased to grade I in 7 patients (58%), grade II in 4 patients (33%), and grade III in 1 patient (8%). In group B, the results seen at 12 months after APC treatment revealed a significantly reduced bleeding intensity (p = .019), to grade I in 3 patients (25%), grade II in 8 patients (67%), and grade III in 1 patient (8%), as compared to the preoperative results. Details of the postoperative bleeding intensity of both groups are given in Fig 2.

In general, the frequency and intensity of nasal bleeding 12 months after APC treatment were significantly reduced in group A as compared to group B (Wilcoxon test, p < .05; Figs 3 and 4).

Overall, 93% of the patients in group A were "satisfied" or even "very satisfied" with the treatment result 12 months after surgery; only 42% of the patients in group B gave these ratings. However, in both groups, more than 90% of the patients were willing to undergo the same operation again. The mean hemorrhage-free time interval during which no nasal bleeding occurred was 8 months in group A (range, 2 weeks to 13 months) and 4 months in group B (range, 1 week to 6 months).

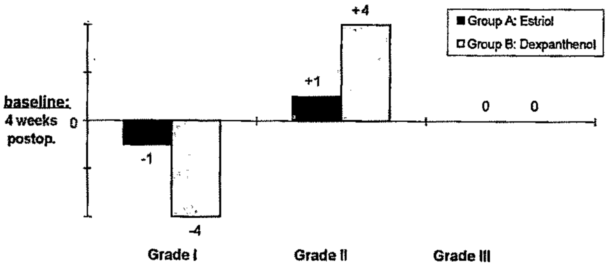

Fig 2. Intensity of bleeding 12 months after surgery, referring to bleeding 4 weeks after surgery as baseline. Indicated is number of patients whose grade of bleeding changed after 12-month assessment. Negative and positive numbers stand for loss or addition of patients in certain grade category of bleeding intensity.

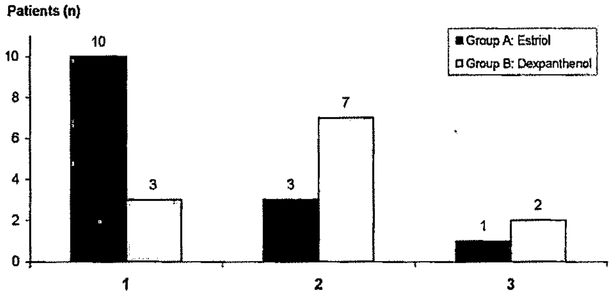

Fig 3. Frequency of bleeding. Grade 1 — less than once per week; grade 2 — several times per week; grade 3 — more than once per day.

DISCUSSION

In a randomized study, we treated 26 patients with known HHT with a combined-therapy regimen of APC and estriol in group A and of APC and dexpanthenol in group B (which was regarded as a standard therapy by the patients' referring physicians).

All patients underwent APC as a primary treatment of the telangiectasias in the nasal mucosa. Argon plasma coagulation offers a variety of advantages. As we have already shown in a previous study,[5] APC is a new surgical procedure that works in a noncontact mode on the tissue surface and provides immediate relief to the patient. Its physical principle is based on the flow of an electrical high-frequency current through ionized argon gas (plasma) from the tip of an applicator. The primarily coagulating effect is at the location of the highest electrical conductivity. Therefore, it is possible to treat even actively bleeding lesions. Furthermore, there is only superficial desiccation and coagulation with limited (1 to 2 mm) penetration — a major factor in minimizing damage to the septal cartilage. No carbonization or vaporization of the tissue is seen during application of the plasma beam. Preliminary studies have shown that this tech-

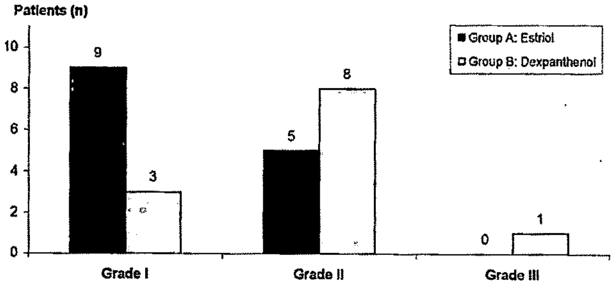

Fig 4. Intensity of bleeding. Grade I — stains on handkerchief; grade II — soaked handkerchief; grade III — bowl or similar utensil necessary.

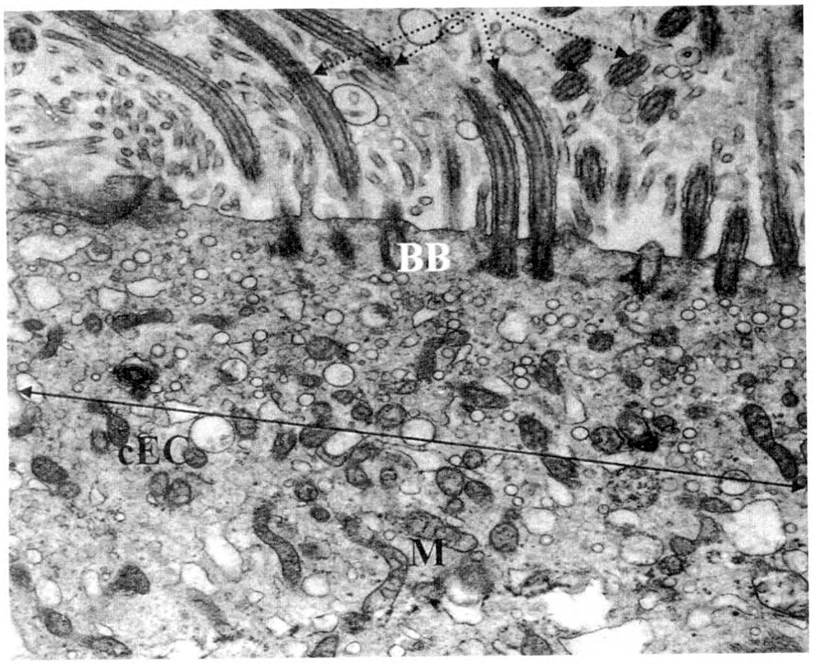

Fig 5. Electron microscopic view of nasal mucosa before treatment with topical estrogens (original ×4,750). Overview of apical cell-half of ciliated epithelial cell (cEC and long arrow) with mitochondria (M) and ciliary basal bodies (BB). Longitudinal and cross-sectional views of dense cilia of epithelial cell (broken arrows).

nique provides a promising low-risk alternative for the treatment of HHT, clearly reducing the severity and frequency of nasal bleeding even in severe cases.[4,5,9,10]

Estrogens have been applied in the treatment of patients with HHT since the 1950s, but so far, only as a systemic treatment method. In 1952, the effectiveness of these hormones in reducing nasal bleeding was first noted by Koch et al.[12] They successfully used estrogen in the form of ethinyl estradiol, which was systemically applied to control epistaxis in both men and women with HHT. Estrogens are thought to prevent severe epistaxis by causing squamous metaplasia, thereby changing the nasal lining from ciliated columnar epithelium to stratified keratinizing squamous epithelium. This thickened lining, covering the telangiectasias, prevents them from being easily affected by local trauma and decreases the vulnerability of the mucosa.[17] However, various studies have described discordant results in the systemic application of estrogens. Oral estrogen therapy was shown to be effective in an uncontrolled study by Harrison,[13] but it was shown to be ineffective in a controlled study by Vase.[17] The use of systemic estrogen therapy remains controversial for the management of epistaxis. The side effects of systemic estrogen therapy carry severe risks that are clearly outlined by Harrison.[13] They include nausea, weight gain, uterine bleeding, breast discomfort and a possible higher risk of developing breast cancer, loss of libido, testicular atrophy, gynecomastia, myocardial infarction, and thromboembolic disease.[18] Many of these side effects are unacceptable to patients even if the side effects can be controlled by stopping the medication or reducing the dosage. Considering the possible risks, the use of systemic hormone therapy remains limited to selected cases.

As a consequence, to avoid systemic side effects, we applied topical estrogens in the nasal mucosa of patients with HHT and recurrent epistaxis. Positive experiences in the use of topical estrogens have already been reported in dermatology and gynecology. In dermatology, topical estrogens have shown an improvement of elastic and collagen fibers by stimulating collagen synthesis of the skin in postmenopausal women.[19,20] In gynecology, topical estrogens are used to reestablish the epithelium, eg, in cases of atrophy of the vaginal mucosa.[21-23]

In our study, we used estriol, a low-potency metabolite of estradiol, for local application on the nasal mucosa. Even in topical estriol application, resorption should be taken into consideration. In our study, 100 mL of ointment contained 100 mg of estriol. We applied, twice a day, approximately 0.5 mL of ointment, which contained 0.5 mg of estriol. Provided a resorption rate of 10%, and considering the pharmacokinetics of estriol,[16] we achieve a theoretical peak serum level of about 25 pg/mL after 2 hours, with a decrease after 10 hours. The physiological range of estriol is known to be 50 to 100 pg/mL; therefore, our calculated peak serum concentrations, which are far below 100 pg/mL, are very unlikely to cause systemic side effects.[16]

For the first time, we used a combined treatment that consists of the surgical reduction of the telangiectasias by the mucosa-preserving APC technique followed by topical estriol treatment of the mucosa

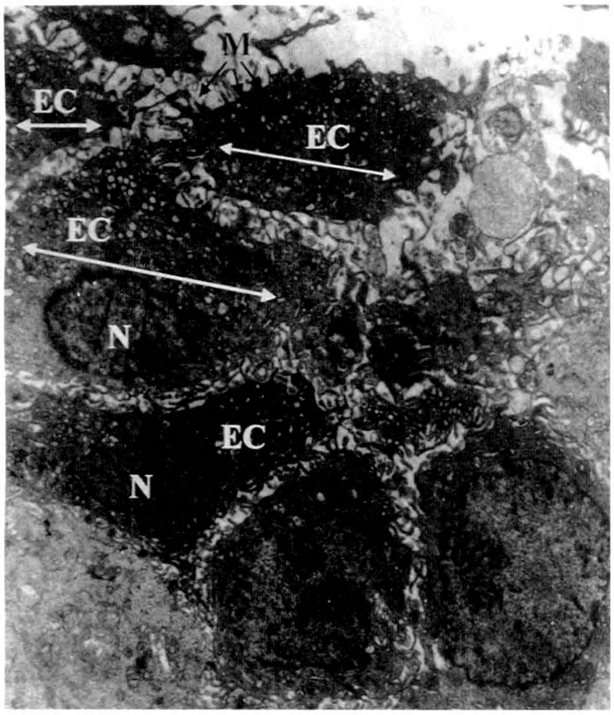

Fig 6. Electron microscopic view of nasal mucosa 3 months after topical estriol application shows change of former ciliated epithelium into incomplete epithelial metaplasia (original ×1,900). Multiple layers of flat epithelial cells (EC and arrows) with loose junctions between them, microvilli (M), and view of nucleus (N) of each epithelial cell.

as a long-lasting, preventive therapy. Patients who regularly applied topical estrogens after APC treatment (group A) showed a significant reduction of the frequency and intensity of nasal bleeding as compared to patients without estriol treatment (group B). Whereas 68% of the patients in group A claimed to have a mild epistaxis with slight stains on the handkerchief (grade I) less than once a week (grade 1), only 25% of the patients in group B obtained these results (Wilcoxon test, $p < .05$).

Histologic and electron microscopic changes of the nasal mucosa were evaluated in 9 of the 14 patients in group A (APC and estriol treatment). Only these patients had previously given a written consent for us to take biopsy specimens from the nasal mucosa. The electron microscopic studies showed, after 3 months of topical estriol application, an incomplete epithelial metaplasia of the nasal mucosa, which changed from a ciliated columnar epithelium to a squamous epithelium (Figs 5 and 6). These changes in the nasal mucosa could not be detected in patients who did not undergo postoperative estriol treatment. In a pilot study, we also observed that the metaplasia of the nasal mucosa is reversible after discontinuation of estriol application. However, we only have these results for a 4-month treatment period.

Because of the metaplasia of the mucosa, one would expect clinical symptoms such as dryness of the nasal mucosa and increased crust formation. The follow-up examinations performed up to 12 months after treatment did not reveal such symptoms in our patients.

A trial with estrogen therapy alone was not intended, as already published studies describing the systemic application of estrogens alone did not show convincing results — not to mention the side effects of systemic estrogen treatment. Already existing telangiectasias of the nasal mucosa must first be removed for benefit to occur from the influence of topically applied estriol during the healing process of the coagulated mucosa.

Using a combined treatment approach, we have shown that topical estriol and APC enable us to significantly prolong the hemorrhage-free time interval and to reduce the severity of bleeding. Until a definite cure is found for HHT and recurrent epistaxis, in our opinion, this combined therapy offers a minimally invasive treatment alternative.

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OCCURRENCE OF FAMILIAL MENIERE'S SYNDROME AND MIGRAINE IN BRASÍLIA

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Since 1941, many families with several members affected by Meniere's syndrome have been described. "Migraine-like" headache was always mentioned. In 1997, we described a large family with Meniere's syndrome and migraine, both transmitted in an autosomal dominant mode. Recently, a gene that could be responsible for Meniere's symptoms has been identified. However, the incidence of familial Meniere's syndrome is still unknown. A. W. Morrison et al estimated this incidence as between 2.9% and 12%. During the years 1997 and 1998, we saw 8 new patients with typical Meniere's syndrome in our outpatient department. They were referred to Dr Messias, who did a careful search for other affected family members. Six of them had relatives with Meniere's syndrome and migraine transmitted in an autosomal dominant mode. The familial incidence of Meniere's syndrome and migraine in Brasília seems to be higher than the figures of Morrison et al for the United Kingdom. The 6 families are described herein.

KEY WORDS — familial incidence, Meniere's syndrome, migraine.

INTRODUCTION

Brown, in 1941[1] and 1949,[2] and Bernstein,[3] in 1965, described families with several members affected by Meniere's syndrome and "migraine-like" headaches. Oliveira and Braga,[4] in 1992, and Oliveira et al,[5] in 1997, described 2 more families with Meniere's syndrome and headache in several family members. Migraine was definitely identified associated with Meniere's syndrome in the 1997 family. Both Meniere's syndrome and migraine appeared to be inherited together as an autosomal dominant trait. All families described showed an autosomal dominant mode of genetic transmission.

Morrison et al,[6] in 1994, studied the relationship between idiopathic Meniere's syndrome (Meniere's disease) and one of the human leukocyte antigens, HLA-C, as well as the presence of enterovirus specific viral protein (VP1) in the peripheral circulation of patients with Meniere's disease. They also tested these relations in families with more than 1 member affected by Meniere's syndrome. They have confirmed both of these associations in sporadic and familial Meniere's syndrome cases. They concluded that the association between Meniere's syndrome and partial class I haplotypes in both sporadic and familial cases points to a Meniere's syndrome locus between the HLA-C and HLA-A loci on the short arm of chromosome 6. The presence of VP1 in the peripheral circulation of Meniere's syndrome patients, which was also confirmed, could be an environmental factor concurring with the production of the symptoms. In 1995, Morrison[7] studied 41 families with more than 1 living member affected by Meniere's syndrome and found the presence of the anticipation phenomenon: in successive generations, the symptoms tend to appear early in life and to be more severe.

Fransen et al,[8] in 1999, studied 1 large Belgian family and 2 small Dutch families with nonsyndromic progressive sensorineural hearing loss and vestibular dysfunction. Linkage studies in the Belgian family mapped the disease to the DFNA9 locus on chromosome 14. Mutation analysis of the COCH gene showed a missense mutation that was exactly the same, described by de Kok et al,[9] in 1999, in a Dutch family with dominant nonsyndromic progressive sensorineural hearing loss: a 208C-T mutation that resulted in a Pro51Ser substitution in the predicted protein. This mutation is also present in a family described by Khetarpal et al,[10] in 1991, with autosomal dominant nonsyndromic sensorineural hearing loss, which is the original DFNA9 family. More than 25% of the patients in the families studied by Fransen et al had episodes of tinnitus, vertigo, aural fullness, and hearing loss. Because of this finding, Fransen et al stated that for the first time a specific genetic mutation in a known gene locus leading to Meniere's disease had been described. However, they point out that 1) the fluctuating hearing loss characteristic of Meniere's disease was not present in the vast majority of their patients and 2) the hearing loss in the affected

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TABLE I. SUMMARY OF AUDIOMETRIC FINDINGS IN EIGHT PROBANDS

Proband	*Age (y)*	*Sex*	*Audiometry*
1	62	M	Bilateral moderate SNHL, downsloping on R, flat on L
2	43	F	Bilateral downsloping SNHL, moderate on R, severe on L
3	30	F	Normal
4	46	F	Normal
5	43	F	Flat moderate SNHL on R, normal on L
6	26	F	Normal
7	45	M	Bilateral moderate SNHL low and high tones, worse on L, middle tones normal
8	35	F	Moderate low-tone SNHL on L, normal on R

Speech discrimination score was compatible with pure tone loss in all patients.

SNHL — sensorineural hearing loss.

patients was in the high tones. No low-tone fluctuating hearing loss was found.

From the studies mentioned above, it is clear that Meniere's disease is being more and more characterized as a genetically determined illness. Some questions, however, remain unanswered: 1) Are migraine and Meniere's disease inherited together, and therefore part of the same symptom complex, as findings in previously described families suggest? and 2) What is the incidence of familial Meniere's disease as opposed to sporadic (idiopathic) cases? Morrison et al,[6] in 1994, stated that the incidence of familial cases lies between 2.9% and 12%, but the latter figure is probably too high, because it is based only on questionnaires.

The first question will be answered by molecular genetics studies on families who display both migraine and Meniere's syndrome. The family we described in 1997 is now being studied. In the years 1997 and 1998, we collected detailed family information on every new patient with typical Meniere's syndrome seen in our outpatient department in an effort to determine how many had other family members affected by the symptoms, including migraine. The results of this effort are reported in this communication.

MATERIALS AND METHODS

During the years 1997 and 1998, every patient seen for the first time in our outpatient department with tinnitus, dizziness, and hearing loss was carefully screened to see whether they fullfilled the criteria set forth by the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology–Head and Neck Surgery for the diagnosis of typical Meniere's syndrome.[11] Briefly, the following criteria were applied: 1) more than 1 episode of spontaneous vertigo that lasted at least 20 minutes and was debilitating and followed by nausea and vomiting without loss of consciousness, 2) documented fluctuating sensorineural hearing loss accompanying the vertiginous episodes, 3) unilateral or bilateral tinnitus during the vertiginous crisis, and 4) the presence of aural fullness, worse during the vertiginous episodes. The presence of headache was not required for the diagnosis of typical Meniere's syndrome.

Patients who fulfilled the above criteria were referred to Dr Messias, who confirmed the diagnosis of Meniere's disease by excluding other possible causes and by performing the following diagnostic tests: 1) laboratory tests, including complete blood count (CBC), blood coagulation tests, cholesterol and triglyceride tests, VDRL (Venereal Disease Research Laboratory) and FTAabs (fluorescent treponema antibody absorption) tests for syphilis, and an electrolyte test, and 2) hearing and vestibular tests, including audiometry with a speech discrimination test, tympanometry, a stapedial reflex test, vectoelectronystagmography (VENG), and a glycerol test.

All patients with an established diagnosis of typical idiopathic Meniere's syndrome (Meniere's disease) were then carefully interviewed by Dr Messias. At this point, the symptoms of headache and migraine were actively searched for in all patients, and when present, they were classified according to the International Headache Classification of 1988. Next, a careful family history was taken in search of other members of the family affected by the symptoms being studied. Pedigrees of each family were constructed, displaying normal and affected siblings of each proband.

Finally, every available member of the families being studied was interviewed, and affected members went through the same diagnostic tests as the probands.

Dr Ferrari, a professor of clinical genetics, went through all of the pedigrees and actively participated in the interviews with all family members.

All probands were required to have typical Meniere's syndrome according to the criteria of the American Academy of Otolaryngology–Head and Neck Surgery, but family members who displayed 2 or more symptoms, including headache or migraine alone, went through the full workup and were enrolled as being affected if clinical, audiometric, and laboratory findings pointed in this direction.

RESULTS

During the study period, 8 patients who displayed

TABLE 2. SUMMARY OF CLINICAL, AUDIOMETRIC, AND VENG FINDINGS IN AFFECTED MEMBERS OF SIX FAMILIES

Probands	*Affected Family Members*	*Age (y)*	*Sex*	*Tinnitus*	*Aural Fullness*	*Fluctuating Hearing Loss*	*Vertigo*	*Nausea*	*Vomiting*	*Audiometry*	*VENG*	*Scotoma*	*Photophobia*	*Atypical Headache*	*Hemicranial Headache*	*Pulsatile Headache*
III-5		30	F	+	+	+	+	+	+	N	PIS R	+	+	–	+	+
(Family 3)	I -2	Deceased	F	+	+	+	+	+	–	ND	ND	+	+	–	+	+
	II-2	60	F	–	–	–	–	+	–	ND	ND	+	+	–	+	+
	III-6	16	F	+	–	+	+	–	–	SNHL bilat	PIS R	+	+	–	+	+
	III-8	37	F	–	–	–	–	+	–	ND	ND	–	+	–	+	+
	IV-1	10	M	+	+	+	+	–	–	N	N	–	–	+	–	–
III-2		46	F	+	+	+	+	+	+	N	PIS R	–	–	+	–	–
(Family 4)	II-2	60	F	+	–	–	+	–	–	N	N	–	+	–	+	+
	II-3	58	F	+	–	–	+	–	–	N	N	+	–	–	+	+
	II-4	65	F	+	+	–	+	+	+	N	PIS L	–	+	–	+	+
	III-3	43	F	–	–	–	+	–	–	N	PIS bilat	+	+	–	+	+
	III-7	33	F	–	–	–	–	–	–	ND	ND	–	+	–	+	+
	III-8	32	F	–	–	–	–	–	+	ND	ND	–	+	–	–	–
	IV-1	25	F	–	–	–	+	+	–	ND	ND	–	–	–	–	–
III-5		43	F	+	+	+	+	+	+	SNHL R	ND	+	+	–	+	+
(Family 5)	III-4	46	F	+	–	–	+	–	–	N	N	–	–	+	–	–
	IV-3	14	F	+	–	+	–	–	–	N	N	–	–	–	–	–
III-9		26	F	+	+	+	+	+	+	N	PIS bilat	+	+	–	+	+
(Family 6)	II-5	75	M	+	–	–	+	+	+	SNHL L	N	–	+	–	+	+
	III-8	32	F	+	–	–	+	–	–	ND	ND	–	+	–	+	+
	II-4	65	F	–	–	–	–	+	–	ND	ND	–	+	–	+	+
	III-2	46	M	–	–	–	–	–	–	ND	ND	–	+	–	–	–
	III-6	36	F	–	–	–	+	+	–	ND	ND	–	+	–	+	+
	II-1	40	F	–	–	–	–	+	–	ND	ND	+	+	–	+	–
	II-2	50	F	–	–	–	–	–	–	ND	ND	+	–	–	+	+
	II-4	52	F	–	–	–	–	+	+	ND	ND	–	+	–	+	+
II-22		45	M	+	+	+	+	+	+	SNHL L	N	–	–	–	–	–
(Family 7)	II-13	62	M	+	+	–	+	+	–	SNHL bilat	ND	+	–	+	+	+
	II-18	58	M	–	–	+	+	–	–	SNHL bilat	ND	–	–	–	–	–
	II-17	56	F	+	–	+	–	–	–	SNHL bilat	ND	+	–	+	+	+
	III-4	20	F	+	+	+	–	–	–	ND	ND	–	+	–	+	+
	III-5	12	F	–	–	–	–	–	–	N	N	–	+	–	+	+
III-5		35	F	+	+	+	+	+	+	N	PIS bilat	+	+	–	+	+
(Family 8)	II-5	54	F	+	–	+	–	+	–	ND	ND	+	+	–	+	+
	II-1	50	F	–	–	–	+	+	–	ND	ND	–	–	–	+	+

Tympanometric and stapedial reflex results were normal in all patients who had them done. Proband 1 had full-blown Meniere's syndrome and atypical headache. Proband 2 had both Meniere's and migraine symptoms.

VENG — vectoelectronystagmography, N — normal, PIS — peripheral irritative syndrome, ND — not done.

all of the symptoms of Meniere's syndrome were seen for the first time in our outpatient department and were enrolled as index patients (probands).

By definition, all probands had full-blown Meniere's syndrome: tinnitus, fluctuating hearing loss, vertiginous attacks, and aural fullness. There were 2 male and 6 female probands. Their ages varied between 26 and 63 years (mean, 43 years). The symptoms started between 15 and 40 years of age in 6 of the 8 probands. Tinnitus and hearing loss were reported in the right ear by 2 patients, in the left ear by 4, and in both ears by 2. Two patients reported tinnitus as the first symptom to appear, followed by vertiginous attacks at least 1 year later. One patient reported vertiginous attacks that preceded tinnitus by 2 years, and 5 said the tinnitus and vertiginous attacks came together. Fluctuating hearing loss appeared with the vertiginous attacks, as well as aural fullness. Seven of the 8 patients had the referred headache associated with Meniere's syndrome, and in 6 of them, the headache was classified as migraine according to the International Headache Classification of 1988. Three patients had migraine crises before the Meniere's symptoms, 2 had migraine attacks after the Meniere's symptoms, and 1 had migraine crises that were unrelated to the Meniere's symptoms. Physical examination findings in the head and neck were normal in all

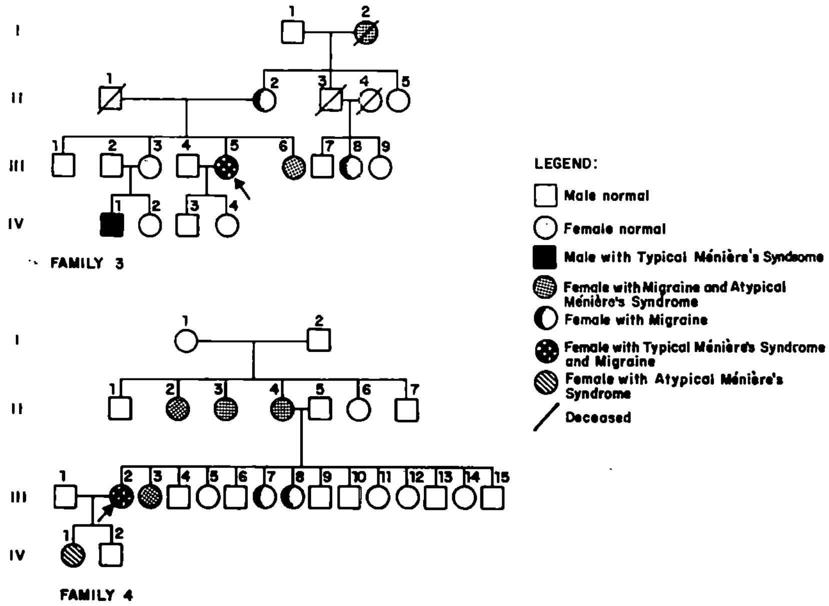
I
II
III
IV
FAMILY 3
FAMILY 4
LEGEND:
Male normal
Female normal
Male with Typical Ménière's Syndrome
Female with Migraine and Atypical Ménière's Syndrome
Female with Migraine
Female with Typical Ménière's Syndrome and Migraine
Female with Atypical Ménière's Syndrome
Deceased

Fig 1. Pedigrees of families 3 (top) and 4 (bottom). Families 1 and 2 are not shown because they had no members other than probands who were affected by Meniere's or migraine symptoms. Arrows indicate probands.

patients, as were the results of tympanometric studies, including stapedial reflex tests. Audiometry (Table 1) performed in intervals between crises showed normal findings in 3 patients and showed sensorineural hearing loss in 5. The hearing losses were in the same ears that had the Meniere's symptoms and varied from moderate low-tone loss to moderate and severe flat sensorineural hearing loss. The VENG, done also between crises, yielded normal findings in 2 cases. Two patients showed peripheral irritative syndrome on the right side, 2 on the left side, and 2 in both ears (Table 2). None of the probands accepted the glycerol test after it was explained to them.

One of the probands (No. 1) does not have other relatives with Meniere's or migraine symptoms. His 5 children are unaffected, and his grandson, although very young, is also unaffected.

Another proband (No. 2) does not know her relatives, because she was raised by an adoptive family. Her 23-year-old son is unaffected. We therefore cannot be sure about the family history of this patient, but for the purpose of this study, we will consider this case as idiopathic or sporadic.

The third proband is female, and her pedigree is shown in Fig 1, top. Her 17-year-old sister (III-6 in Fig 1, top, and Table 1) has typical migraine symptoms that precede vertiginous attacks with bilateral tinnitus and fluctuating hearing loss. Audiometry performed between crises showed moderate bilateral sensorineural hearing loss at 6 and 8 kHz, and VENG showed bilateral peripheral irritative syndrome. A 10-year-old nephew (IV-1 in Fig 1, top, and Table 2) has crises of vertigo, bilateral tinnitus, and aural fullness. His audiometric results are normal, and he has no headache. Her mother (II-2 in Fig 1, top, and Table 2) has typical migraine symptoms. Her grandmother (I-2 in Fig 1, top, and Table 2) had migraine symptoms accompanied by vertiginous attacks and aural fullness. One cousin (III-8 in Fig 1, top, and Table 2) has symptoms compatible with migraine only.

The fourth proband is female, and her pedigree is displayed in Fig 1, bottom. Two of her sisters (III-7 and III-8 in Fig 1, bottom, and Table 2) have typical migraine, and another (III-3) also has dysequilibrium that follows the migraine crises. The VENG showed bilateral peripheral irritative syndrome. The audiometric findings were normal for III-3, and audiometry was not done for III-7 and III-8. The proband's mother (II-4 in Fig 1, bottom, and Table 2) has vertigo, tinnitus, and aural fullness in the left ear, pulsatile headache with nausea, and photophobia. Audiometry showed bilateral sensorineural hearing loss that was moderate and in the high frequencies. The VENG showed peripheral irritative syndrome on the left. Two of her mother's sisters (II-2 and III-3 in Fig 1, bottom, and Table 2) have vertiginous attacks and tinnitus associated with pulsatile headache. One daughter (IV-1 in Fig 1, bottom, and Table 2) has vertiginous attacks, but unfortunately, refused further workup.

The fifth proband is also female, and her pedigree is displayed in Fig 2, top. One sister (III-4 in Fig 2, top, and Table 2), 46 years old, has typical migraine associated with vertiginous attacks and tinnitus without fluctuating hearing loss and aural fullness; her audiometric findings were normal. One daughter (IV-3 in Fig 2, top, and Table 2), 14 years old, has had tinnitus and fluctuating hearing loss for the past 2 years. She has no vertigo, and her audiometric find-

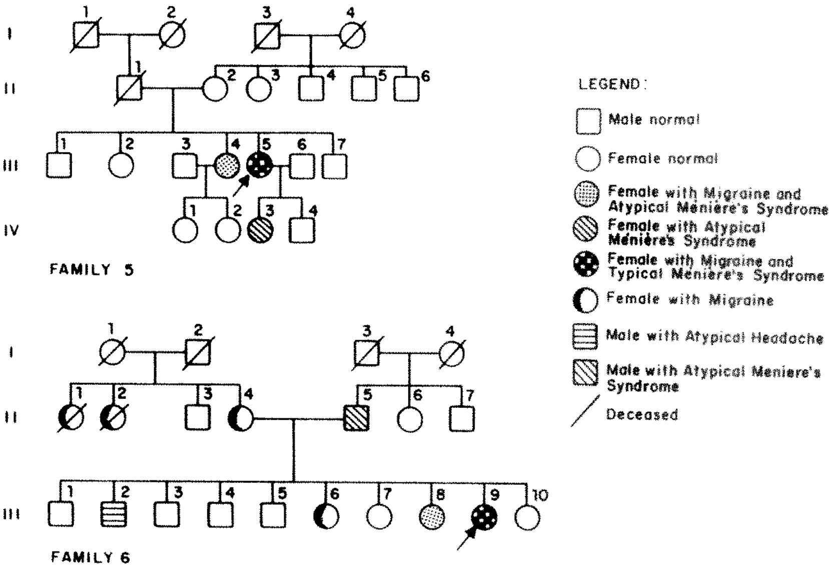
FAMILY 5
FAMILY 6
I
II
III
IV
LEGEND:
Male normal
Female normal
Female with Migraine and Atypical Ménière's Syndrome
Female with Atypical Ménière's Syndrome
Female with Migraine and Typical Ménière's Syndrome
Female with Migraine
Male with Atypical Headache
Male with Atypical Meniere's Syndrome
Deceased

Fig 2. Pedigrees of families 5 (top) and 6 (bottom).

ings were normal.

The sixth proband is also female, and her pedigree is shown in Fig 2, bottom. Her mother (II-4 in Fig 2, bottom, and Table 2) has typical migraine symptoms without vertigo, and her father (II-5 in Fig 2, bottom, and Table 2) has vertiginous attacks with tinnitus and nausea. Audiometry of the father showed moderate sensorineural hearing loss in the left ear, and his VENG results were normal. Two aunts (II-1 and II-2 in Fig 2, bottom, and Table 2) had typical migraine symptoms. One brother (III-2 in Fig 2, bottom, and Table 2) has atypical headache that seems to be of vascular origin. One sister (III-6 in Fig 2, bottom, and Table 2) has typical migraine symptoms, and another sister (III-8 in Fig 2, bottom, and Table 2) has vertiginous attacks with unilateral tinnitus preceding typical migraine symptoms.

The seventh proband is male, and his pedigree is shown in Fig 3, top. His 62-year-old brother (II-13 in Fig 3, top, and Table 2) has typical migraine that is unrelated to vertiginous attacks and tinnitus plus aural fullness that may occur twice in one day. Audiometry showed moderate low-frequency sensorineural hearing loss in the right and left ears. One sister (II-17 in Fig 3, top, and Table 2) has had tinnitus and hearing loss in the right ear, as well as vertiginous attacks, for the past 20 years. Typical migraine symptoms precede the vertiginous attacks. Audiometry showed bilateral moderate sensorineural hearing loss. Her 20-year-old daughter (III-4 in Fig 3, top, and Ta-

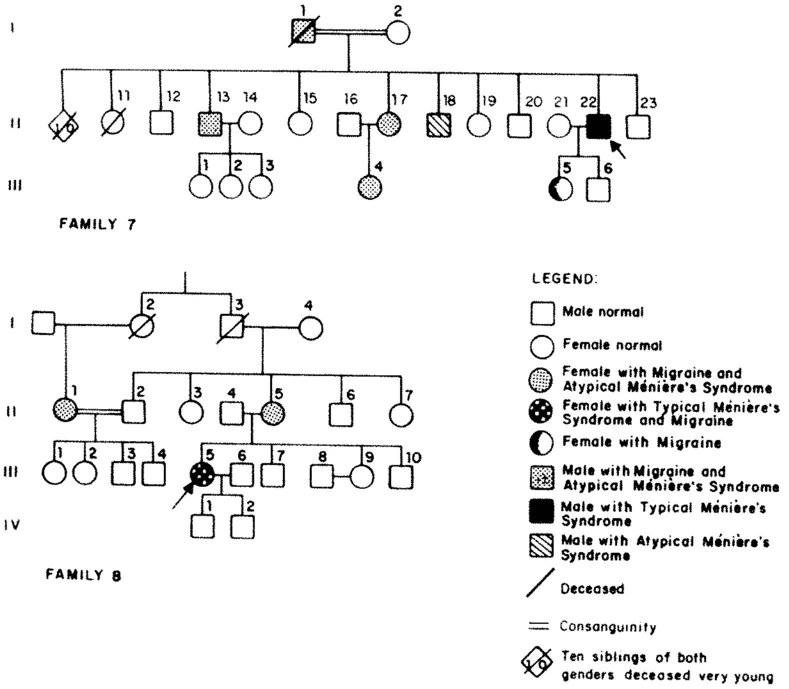
FAMILY 7
FAMILY 8
I
II
III
IV
LEGEND:
Male normal
Female normal
Female with Migraine and Atypical Ménière's Syndrome
Female with Typical Ménière's Syndrome and Migraine
Female with Migraine
Male with Migraine and Atypical Ménière's Syndrome
Male with Typical Ménière's Syndrome
Male with Atypical Ménière's Syndrome
Deceased
Consanguinity
Ten siblings of both genders deceased very young

Fig 3. Pedigrees of families 7 (top) and 8 (bottom).

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ble 2) has had typical migraine symptoms for the past 14 years. For the past 11 years, she has had bilateral tinnitus, plus fluctuating sensorineural hearing loss and aural fullness in the left ear. Vertiginous attacks occur during the migraine crises. She was interviewed by telephone, but could not come for a full workup. Another brother (II-18 in Fig 3, top, and Table 2) has had vertiginous attacks and fluctuating hearing loss in the right ear for the past 2 years. He has sensorineural hearing loss, severe in the right ear and moderate in the left. He reported typical migraine symptoms that began when he was 24 years old and disappeared 5 years ago. He is now 56 years old. The proband's father (I-1 in Fig 3, top, and Table 2) had headache and vertigo, but is no longer alive. The proband's daughter (III-5 in Fig 3, top, and Table 2) is now 12 years old and for 1 year has had hemicrania that worsens with intense light.

The eighth proband is female, and her pedigree is shown in Fig 3, bottom. Her 54-year-old mother (II-5 in Fig 3, bottom, and Table 2) has had typical migraine symptoms for the past 17 years, occurring almost daily. Tinnitus and hearing loss appeared 1 year ago in the left ear. Her mother's cousin and sister-in-law (II-1 in Fig 3, bottom, and Table 2), who is 50 years old, has hemicrania that is pulsatile, insidious, and accompanied by photophobia and nausea. Three years ago, she had 1 episode of vertigo with nausea but without tinnitus or hearing loss.

Table 2 summarizes the clinical, audiometric, and VENG findings in the probands and all affected family members.

DISCUSSION

In order to avoid any doubt about the presence of Meniere's syndrome in the probands, we have enrolled only patients with the full-blown syndrome. To confirm the presence of Meniere's disease (idiopathic Meniere's syndrome), we excluded all known causes of Meniere's syndrome (syphilis, head trauma, perilymphatic fistula) by careful history-taking and audiometric, laboratory, and VENG studies. Once we confirmed the diagnosis of Meniere's disease, we applied the questionnaires for migraine and family history.

The fact that only 8 new patients with Meniere's disease were seen during the years 1997 and 1998 in our outpatient department suggests that this disease is far less frequent in central Brazil than in Europe or North America. Indeed, Maudonnet et al,[12] in 1998, reported on 4,825 patients with vestibular complaints seen in the Department of Otolaryngology of the State University of Campinas, São Paulo, Brazil, and only 5% were found to have Meniere's disease, including atypical or incomplete presentations. In Brasília, we do not have complete records of all patients with Meniere's disease, but over the past 23 years (1977 to 1999), only 16 patients underwent operation (endolymphatic sac enhancement procedure) for Meniere's disease in our department. On the other hand, in 6 of the 8 patients, we were able to demonstrate a familial incidence of symptoms, and 6 of the 8 probands also had migraine symptoms.

It is not possible, from our data, to make any final statement about the incidence of familial Meniere's disease in Brasília, because of the small number of patients studied, and because no formal epidemiological study was performed on our whole population. Certainly, however, our results suggest that the familial incidence is much higher than the one suggested by Morrison et al[6] for the United Kingdom.

Morrison,[7] in 1995, stated that Meniere's disease is rare in blacks and American Indians and predominant in whites. The population of central Brazil has a large component of people of mixed black, Indian, and white background and a smaller component of pure Caucasians, and this fact may explain the lower incidence of Meniere's disease than in European and North American populations. Indeed, 7 of the 8 probands were white. The age of onset of symptoms in our patients is comparable to that described by Paparella[13] in 1985, and the higher incidence of the disease in women that is well described in the literature is also present in our patients.

The audiometric results displayed in Table 1 for the 8 probands show sensorineural hearing loss in 5 probands, 3 of whom have bilateral losses, and normal hearing in 3 probands. All 3 patients with normal audiometric results did complain of hearing loss during the attacks, but the fluctuation in hearing could not be documented. The VENG findings were normal in 2 probands and showed peripheral irritative syndrome in 6 probands, bilateral in 2 cases. These results are compatible with Meniere's disease. Unfortunately, the glycerol test and electrocochleography are not easily accepted by volunteers in Brazil.

Inspection of the pedigrees of the 6 families (Figs 1-3) shows an autosomal dominant mode of genetic transmission in all of them. In family 7, there is a history of consanguinity of the proband's parents, but the presence of the symptoms in successive generations strongly suggests an autosomal dominant mode of genetic transmission. Because the migraine and Meniere's symptoms are not always complete in all family members, we can infer that the penetrance is variable, and because in family 3 (Fig 1, top) the proband's sister (III-3) has no symptoms, while her son has typical Meniere's syndrome, we can infer that incomplete penetrance may be present. In family 8 (Fig 3, bottom), a brother and a sister (I-2 and I-3) who are unaffected have affected daughters (II-1 and II-5). Patient II-1 has 2 daughters (III-1 and III-2) and 2 sons (III-3 and III-4) who are unaffected, while patient II-5 is the proband's mother. Autosomal dominant genetic transmission with incomplete penetrance is again suggested. The anticipation phenomenon described by Morrison[7] in 1995 is present in family 3 (Fig 1, top): the son of the proband's sister (IV-1, Fig 1, top) is 10 years old and has all of the Meniere's symptoms. The same phenomenon is present in family 5 (Fig 2, top): the proband's 14-year-old daughter has atypical Meniere's syndrome.

Table 2 summarizes the clinical, audiometric, and VENG findings in the probands and all affected family members. Laboratory, tympanometric, and stapedial reflex test results were omitted, because they were all normal. Many patients who have only migraine are young, and they may develop Meniere's symptoms later.

It is important to point out the presence of migraine in 6 of the 8 probands, as well as the positive family history for this syndrome in all 6 families (Figs 1-3 and Table 2). In 5 probands, migraine either preceded or followed the Meniere's symptoms, and in 1, the migraine crisis was independent of the Meniere's symptoms. It appears, therefore, that as pointed out by Parker[14] in 1995, Oliveira et al[5] in 1997, and Morrison[15] in 1981, as well as Prosper Meniere[16-18] in 1861, migraine is a part of the Meniere's symptom complex. Our data suggest that migraine is a variable expression of Meniere's disease and that both are inherited together in an autosomal dominant mode.

In a 1992 article,[4] we mentioned that a central cause for Meniere's disease had been postulated by some authors.[19-22] "Migraine-like" headaches would occur as a consequence of microvascular changes in the central nervous system. Through the autonomic nervous system, the microcirculation of the inner ear would be affected, leading to the appearance of Meniere's syndrome and endolymphatic hydrops. In a 1997 article,[5] we showed that indeed, the "migraine-like" headache that accompanies the Meniere's symptoms is true migraine. We stated then that this finding favors the above-mentioned theory. Now we have shown that migraine is inherited together with Meniere's syndrome monogenically. This finding further enhances the idea of central microvascular changes as the initiating event for Meniere's disease. Whether the vascular changes in the inner ear occur in the endolymphatic sac is a matter for further investigation.

Morrison et al,[6] in 1994, investigated the possibility of a viral infection's being the environmental factor that leads to the occurrence of Meniere's disease in genetically predisposed patients and families. Genetically determined receptors in the neurons and inner ear cells could facilitate the viral infection. Herpes simplex virus inner ear infection is a possibility in this context.

In conclusion, we can say the following.

1. The incidence of Meniere's disease in central Brazil is probably much lower than that in Europe and North America. Differences in genetic background probably explain this finding.

2. The occurrence of a positive family history for Meniere's disease seems to be much higher in central Brazil (6 of 8 randomly selected patients) than the 2.9% to 12% occurrence estimated by Morrison et al[6] for the United Kingdom.

3. Meniere's disease and migraine appear to be inherited together in an autosomal dominant mode with variable expressivity and incomplete penetrance. The anticipation phenomenon present in our families speaks in favor of an autosomal dominant mode of genetic transmission.

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VASCULAR OCCLUSION IN THE ENDOLYMPHATIC SAC IN MENIERE'S DISEASE

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In 2 patients with severe Meniere's disease (MD), there was histologic evidence of occlusion of the vein of the vestibular aqueduct (VVA). This finding coincided with total or partial occlusion of numerous small vessels around the endolymphatic sac (ES), flattening of epithelium, extensive perisaccular fibrosis, and signs of new bone formation. Ultrastructural analysis of the occluding material showed foci with dense connective tissue, calcification, lipid deposits, and layers of basement membrane, sometimes concentrically arranged. The exact nature of the occluding material was unknown. In another 2 MD patients, the VVA was not visualized, and the ES vessels showed no signs of occlusion. Seven controls with acoustic schwannoma or meningioma had normal vasculature. The presence of vascular impairment in the ES in MD patients indicated that altered hemodynamics may contribute to the pathogenesis of endolymphatic hydrops and MD.

KEY WORDS — inner ear, Meniere's disease, ultrastructure, vascular occlusion.

INTRODUCTION

The endolymphatic duct (ED) and endolymphatic sac (ES) are believed to be involved in endolymph homeostasis. Absorption, secretion, and enzymatic degradation of endolymph components may be important in the process of volume-pressure regulation of the inner ear and inner ear fluid balance.[1-4] Their functional level could be reflected in the high metabolic activity of the ED and ES, which, according to Koburg et al,[5] was equal to or higher than that of the stria vascularis and planum semilunatum. It has been shown that an extensive vascular plexus surrounds the ED and ES.[6]

Vasomotor disturbances affecting the vessels of the inner ear were early proposed as a cause of Meniere's disease (MD).[7-11] Bomholt[12] found hypofibrinolysis in some MD patients, suggesting an inner ear vascular disorder causing symptoms of MD and endolymphatic hydrops. A decreased microvascular circulation in the ES, including impaired venous drainage from the ED and ES through the vein of the vestibular aqueduct (VVA), may lead to morphological changes that alter normal ES function.[13,14] Malfunction in the vasculature surrounding the ED and ES may theoretically result in impaired regulation of fluid balance and disturbance of inner ear pressure control.

The difficulty of obtaining temporal bone specimens from MD patients accounts for the sparse knowledge of the histopathology of the ED and ES in this disease. During labyrinthectomy for severe MD in 4 patients, we removed the vestibular aqueduct (VA), including a thin shell of surrounding bone. In 1 patient, the ED and adjacent accessory canal (AC) with the VVA at this level were included in the specimen, and in a second patient, the AC with the VVA was seen at the level of the proximal ES. In the remaining 2 patients, the AC was not included in the specimens. All patients had undergone previous ablative therapy to control vertigo by ultrasound irradiation, transtympanic gentamicin instillation, or both (see Table).

MATERIALS AND METHODS

Patient 1. A 64-year-old man had had left-sided MD since the age of 49. Initial audiometry revealed bilateral sensorineural hearing loss at high frequencies, more pronounced in the left ear, in which a fluctuant low-tone loss was also evident. A glycerol test proved positive in the left ear at this stage. At the age of 56, the patient had experienced 3 episodes per year of vertigo lasting 2 to 4 hours. One year later, he suffered 7 spells of disabling vertigo in 1 month. At age 59, surgical ultrasound irradiation directed at the vestibular part of the inner ear was carried out. This was repeated 2 years later, and the patient remained well until the age of 63, when severe attacks of vertigo and vomiting recurred. At this stage, because the patient had a flat-loss audiogram with a pure tone average (PTA) of 90 dB, total labyrinthectomy was performed (see Table). The patient made a

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MORPHOLOGY OF ENDOLYMPHATIC SAC IN PATIENTS WITH MENIERE'S DISEASE AND PATIENTS WITH SCHWANNOMA OR MENINGIOMA

Patient No.	*Sex*	*Age (y)*	*Treatment*	*Vein of Vestibular Aqueduct*	*Endolymphatic Sac Vasculature*	*Stainable Luminal Substance*	*Epithelial Flattening*	*Subepithelial Fibrosis*	*New Bone Formation in Vestibular Aqueduct*
Meniere's disease									
1	M	64	Ultrasound ×2, labyrinthectomy	Partly occluded	Partly occluded*	Moderate	Slight	Moderate	Moderate
2	M	45	Ultrasound ×2, gentamicin intratympanically, labyrinthectomy	Not known	Unaffected*	Moderate	Slight	Slight	Slight
3	F	46	Ultrasound ×2, gentamicin intratympanically ×2, labyrinthectomy	Not known	Unaffected*	Moderate	Slight	Moderate	None
4	F	42	Gentamicin intratympanically	Partly occluded	Partly occluded*	Sparse	Extensive	Extensive	Extensive
Schwannoma									
5	F	26	Translabyrinthine surgery, 27 mm†	Open	Unaffected*	Abundant	None	None	Slight
6	M	21	Translabyrinthine surgery, 30 mm†	Open	Unaffected*	Sparse	None	None	None
7	F	67	Translabyrinthine surgery, 30 mm†	Open	Unaffected*	None	None	None	None
8	F	60	Translabyrinthine surgery, 20 mm†	Open	Unaffected*	None	None	None	None
9	M	58	Translabyrinthine surgery, 25 mm†	Open	Unaffected*	Sparse	None	None	Slight
10	M	48	Translabyrinthine surgery, 27 mm†	Open	Unaffected	Sparse	None	None	None
Meningioma									
11	F	49	Translabyrinthine surgery, 27 mm†	Open	Unaffected	Very sparse	None	None	None

*Ultrastructural analysis.
†Diameter of extrameatal portion.

quick recovery and had no further spells of vertigo. A detailed history and pathology report of this patient was published earlier.[15]

Patient 2. A 45-year-old man had had left-sided MD since the age of 39. Initial audiograms showed fluctuant low-tone and high-tone loss in the left ear. At the age of 42, he experienced 2 vertigo episodes per month, lasting 5 to 7 hours. The attacks appeared without an aura. At the age of 43, when the patient had a flat-loss audiogram with a PTA of 60 dB, a surgical ultrasound irradiation was carried out. This surgery was repeated 6 months later, and the patient remained well for 10 months. Gentamicin was given 3 times transtympanically at 44 years of age, when attacks of vertigo and vomiting recurred. A flat-loss audiogram with a PTA of 80 dB was recorded. One year later, because of several 10-minute attacks each day, total labyrinthectomy was performed (see Table). The patient made a quick recovery and had no further spells of vertigo.

Patient 3. A 46-year-old woman had had right-sided tinnitus since the age of 20. At 39 years, the hearing deteriorated in her right ear after several attacks of vertigo. Fluctuations in low-tone hearing were observed, and a glycerol test proved positive. At the age of 43, she underwent ultrasound surgery because of having a 3- to 12-hour-long vertigo attack every week. This operation was repeated 1 year later because of recurrent monthly vertigo attacks. At the age of 45, the vertigo attacks reappeared, and gentamicin was given transtympanically 3 times, but the spells reappeared 3 months later. Additional transtympanic gentamicin was given twice. At 46 years of age, and a few months after the last gentamicin treatment, vertigo reappeared in the form of drop attacks, and in between attacks, the patient experienced severe dysequilibrium. At this stage, when the patient had a flat-loss audiogram with a PTA of 70 dB, total labyrinthectomy was performed (see Table). The patient made a quick recovery and had no further spells of vertigo.

Patient 4. A 42-year-old woman had had left-sided

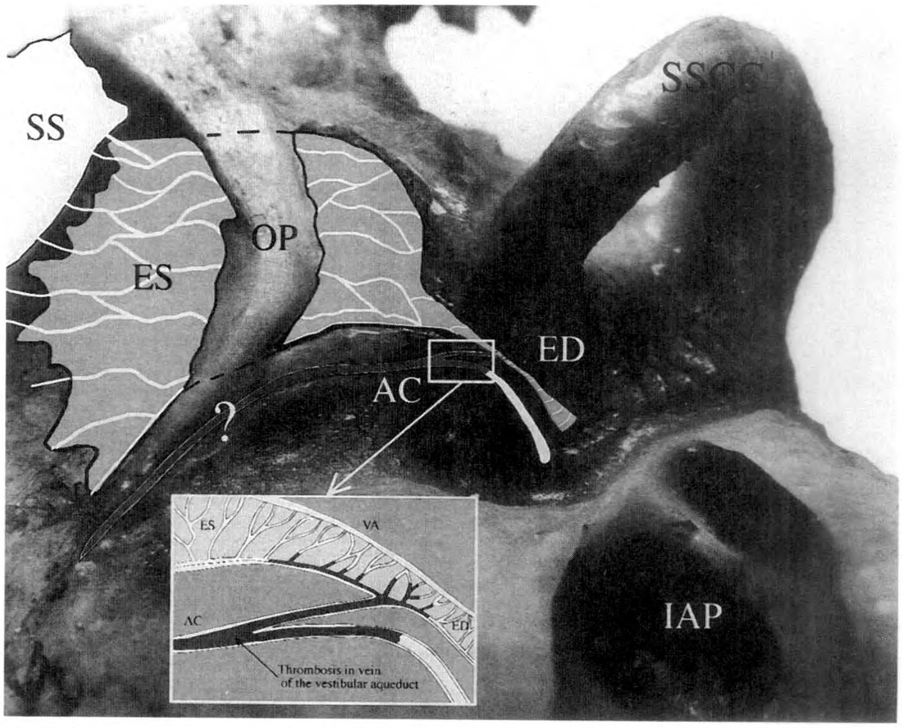


Fig 1. Microdissection of human left temporal bone, medial view. Location of endolymphatic duct (ED) and endolymphatic sac (ES) and site of occlusion in vein of vestibular aqueduct (VVA) are graphically reconstructed. Small bridge of bone (or operculum; OP) was spared to allow visualization of external aperture of aqueduct. **Inset)** Graphical reconstruction of location of occluded vessels and level of proximal part of occlusion in VVA. AC — accessory canal; SS — sigmoid sinus; IAP — internal acoustic porus; SSCC — superior semicircular canal; ? — distal segment of AC was not visualized in serial sections and hence occlusion of distal VVA could not be established.

MD since the age of 39. Initial audiometry revealed a peak-formed audiogram with low- and high-tone sensorineural hearing loss to 40 to 60 dB in the left ear, but normal hearing in the right ear. A glycerol test proved negative in the left ear 2 weeks later, but at this stage, her hearing had improved for the low frequencies. At the age of 40, the vertigo spells became more frequent. Two years later, the patient suffered severe spells of vertigo every day or every other day. Diuretic medication did not alleviate the symptoms. Assessment of hearing in the left ear showed a flat-loss audiogram with a PTA of 80 dB. At this stage, gentamicin was given transtympanically on 3 consecutive days. The vertigo spells disappeared, but reappeared 3 months later in the form of 3 attacks each week. Because her remaining hearing was of no practical use to the patient, total labyrinthectomy was performed (see Table). The patient made a quick recovery and had no further spells of vertigo.

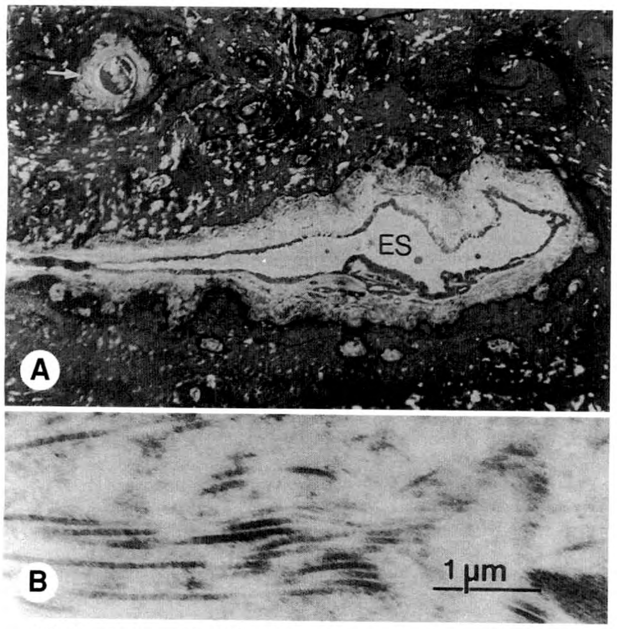
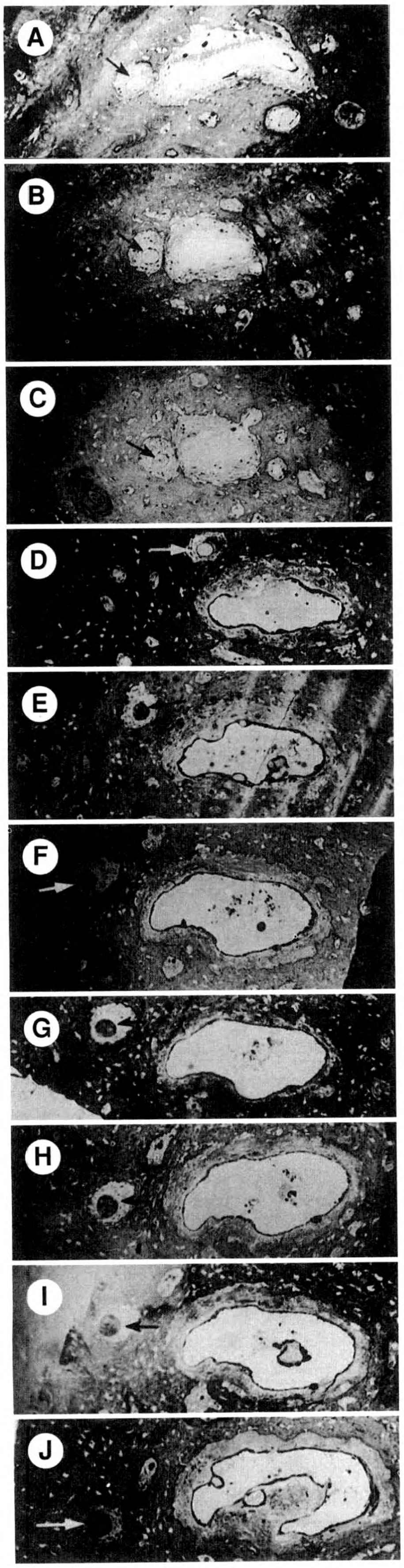


Fig 2. (Patient 1) **A)** Light micrograph of proximal part of endolymphatic sac (ES; original ×100). Note obliterated vein of vestibular aqueduct (arrow). **B)** Bundles of collagen microfibrils in subepithelial tissue show signs of fibrosis (original ×21,000).

Acoustic Neuroma Patients. During translabyrinthine surgery for acoustic neuroma, 18 ES specimens were removed. The ED and AC were included in 7 of these specimens, which were used for comparison with the MD specimens.

Specimen Processing. During labyrinthectomy for MD and translabyrinthine surgery for acoustic neuroma, the entire labyrinthine block was drilled away. The VA was identified near the external aperture in the posterior cranial fossa. The VA was skeletonized with small diamond burs, with only a thin shell of bone left around its lateral surface and the superior and inferior margins. The VA was followed toward its interior opening in the vestibule. At the operculum, the drilling proceeded just proximal to the bony opening into the posterior cranial fossa. When the dura was approached, the whole specimen could be raised to leave the dura of the posterior cranial fossa with the extraosseous ES portion intact. The specimen was immediately immersed in cold buffered fixative containing glutaraldehyde 2.5%, formaldehyde 1.5% in a 0.1 mol/L sodium-phosphate solution. The



bony shell was further thinned and then submitted to 4 months of decalcification in sodium–ethylenediaminetetraacetic acid solution. The tissue block was processed for light microscopy and transmission electron microscopy according to routine methods. Thin sections were viewed in a JEOL electron microscope (JSM 100 SX).

RESULTS

Occlusion or partial occlusion of the VVA was seen in 2 of the 4 MD patients (Nos. 1 and 4; Fig 1). In patient 1, the AC was present in the specimen in the region of the proximal ES, and the contained VVA showed partial occlusion (Fig 2A). In patient 4, in whom the ED and proximal ES were serially sectioned, it was possible to locate the level of the proximal end of the occlusion in the VVA. In this patient, an open lumen of the VVA was revealed in the distal ED and the junction to the proximal ES portion. The proximal end of the occlusion was encountered 200 μm distal to the isthmus of the VA (Figs 1 and 3). The VVA remained occluded 800 μm further distally, as long as the AC could be visualized in the serial sections. The exact length of the obliterated part of the VVA was not established in any of the patients. In the remaining 2 patients, the AC was not present in the specimens, and hence the condition of the VVA could not be ascertained. No artery was seen adjacent to the VVA in any of the patients. Occluded or partially occluded subepithelial, perisaccular, and periductal vessels coincided with the occlusion of the VVA in patients 1 and 4 (Figs 4-6), whereas patients 2 and 3 showed normal microvasculature. All 4 ES specimens from the MD patients showed marked thickening and fibrosis of the perisaccular tissue (see Table). At the ultrastructural level, this corresponded to areas with thick bundles of collagen fibers (Fig 2B). The epithelial cells were generally flattened, and there were scarcely any convolutions or polyps. Signs of new bone formation were seen along the walls of the VA (Fig 4). The luminal contents of the proximal ES showed sparse amounts of stainable homogeneous substance (Fig 4). In patient 1, macrophages and mononuclear cells formed aggregations in the luminal space.

Ultrastructural analysis of the occluded VVA in patient 4, at a level approximately 300 μm distal to the isthmus, revealed that the endothelial layer was

← **Fig 3.** Light micrographs of serially sectioned endolymphatic duct and sac in patient 4 (original ×78). Narrow or isthmus region at transition between duct and sac is seen in **B** and **C**. Lumen of vein of vestibular aqueduct remained open proximally (**A-D**, arrows), but was obliterated distally (**E-J**, arrows). Proximal part of occlusion was located around 200 μm from isthmus region.

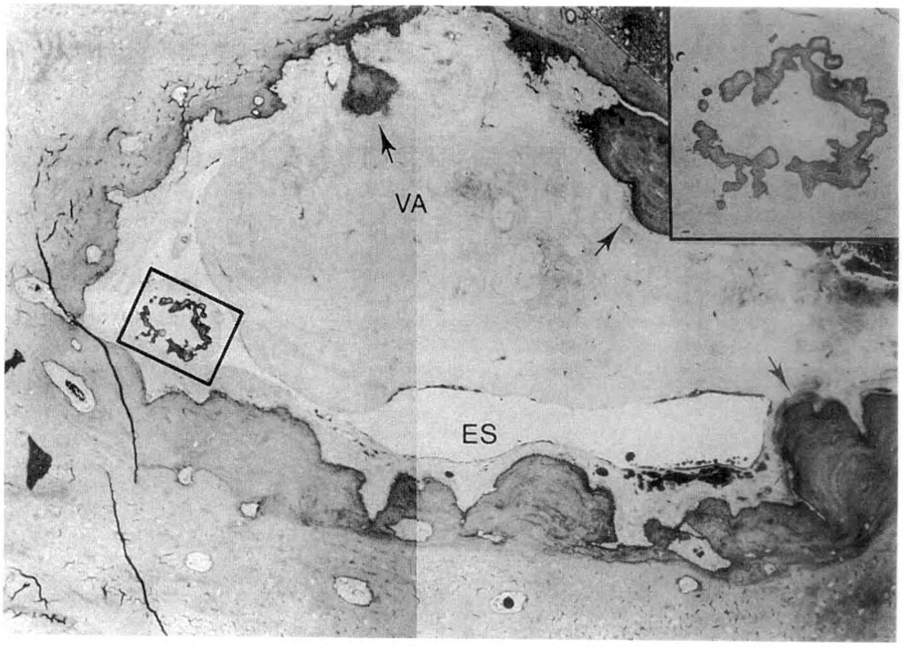


Fig 4. Light micrograph of proximal sac (ES) region in patient 4, around 300 μm distal to isthmus (original ×150). Vestibular aqueduct (VA) is filled with dense fibrous tissue with some obliterated vessels. There are signs of dystrophic calcification of fibrous tissue (framed area), together with new bone formation (arrows). Epithelial lining is thin. Luminal contents are clear. **Inset)** Framed area, showing dystrophic bone formation in higher magnification (original ×400).

flattened and that occluding material filled the entire luminal space. The matrix filling the lumen consisted of dense amorphous fibrous material interspaced with slender foci of darkly staining thrombotic residual deposits together with areas of calcification. At this level, the diameter of the VVA was 45 × 51 μm (Fig 7). Around 50 μm further distally, the VVA was slightly wider, with a diameter of 73 × 84 μm. At this level, the occluding material appeared less dense, and areas of calcification were less prominent (Fig 8). Layers of basal membrane, at times concentrically arranged (Fig 9), and lipid deposits (Fig 9) were sometimes seen in the occluding material in some of the smaller perisaccular vessels. None of the acoustic neuroma patients showed occlusion of the VVA or other vascular abnormalities at the level of the ED and proximal ES. The data on the luminal contents, epithelial and subepithelial layer, and VA are summarized in the Table.

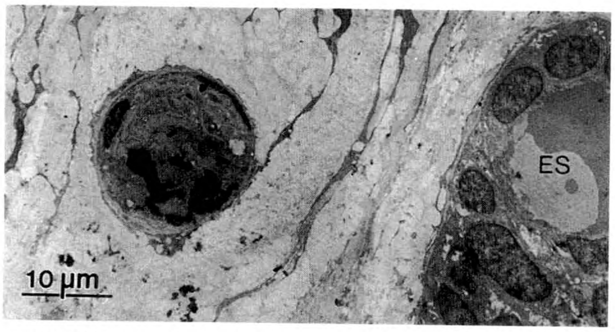


Fig 5. Transmission electron microscopic (TEM) image from patient 1 shows small subepithelial occluded capillary surrounded by subepithelial fibrotic connective tissue (original ×2,000). Intravascular contents appear as mass of organized amorphous material with some darkly staining residua. ES — endolymphatic sac.

DISCUSSION

Vascular disturbances in the ED and ES vessels have been pointed out as a possible factor in the pathophysiology of MD. The present study was the first to demonstrate changes in the vessels of the ED and ES in 2 MD patients. The findings were made in fresh ED and ES biopsy specimens from MD patients who underwent labyrinthectomy in an acute stage of the disease, but not in a group of patients with acoustic neuroma or meningioma. Whether occlusion of the perisaccular vessels and impaired venous drainage through the VVA was the primary cause, which in the long run would lead to clinical MD, or whether the occlusion was the result of an inner ear injury, eg, degeneration of the sensory epithelium, could not be determined from the present data.

Sac ischemia has been shown to produce endolymphatic hydrops in an animal model.[14] The intermediate portion of the ES was affected with flattening of

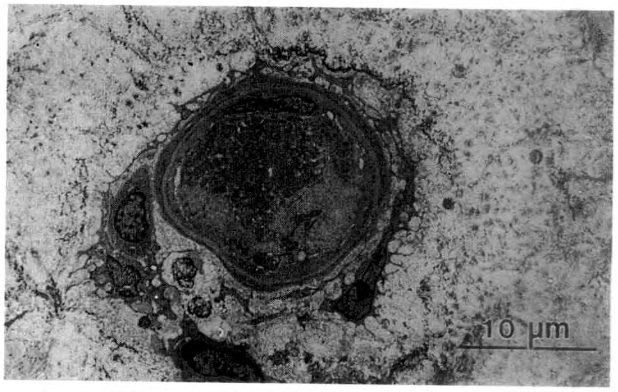


Fig 6. TEM image of small periductal bone channel from patient 1 containing occluded capillary (original ×2,800). Lumen is filled with mass of organized tissue.

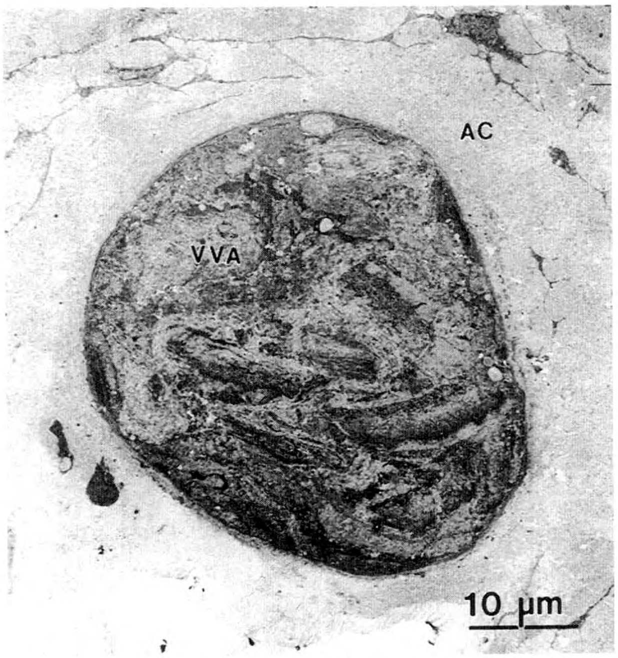


Fig 7. TEM image of occluded vein of vestibular aqueduct (VVA), around 300 µm distal from isthmus (patient 4; original ×1,600). Lumen is totally occluded by amorphous fibrillary material, which presents with different densities. AC — accessory canal.

epithelial cells and a decrease in rugose formation. A reduction of the homogeneous substance in the proximal ES lumen was also noted, indicating a malfunction in the ES epithelial lining.[14] Interference with vascular drainage from the inner ear by blocking the VVA has been found to produce vertigo and partial vestibular and cochlear sensory cell degeneration in gerbils.[16] Endolymphatic hydrops, however, did not appear in these animals.[16]

Increased venous pressure caused by obstructive vascular changes along the ES and the VVA, resulting in insufficient drainage of the vestibular organs, has been suggested to be a crucial mechanism in the pathophysiology of MD.[13] Shambaugh[17] was impressed by the "bloodless appearance" of the sac wall during a shunt operation in an MD patient. Increased venous flow and pressure could also be the result if the VVA received additional veins, normally tributaries of the posterior vestibular vein, as described by Mazzoni[18] in 25% of his patients. Endolymphatic hydrops was seen in a patient who lacked the VVA, probably because of a developmental anomaly.[19] In this patient, however, there was no vertigo.[19] Ikeda and Sando[20] also found partial absence of the VVA in 15% of temporal bones from individuals with endolymphatic hydrops and MD. They also observed decreased perisaccular vascularity in patients with MD.[21] Although they recognized the importance of the VVA in the absorption of endolymph in the ES, they thought that it played no major role in the pathogenesis of MD.[20] A radiographic study showed dehiscence of the distal VA produced by a high jugular fossa in 4 of 6 patients with MD-like symptoms.[22] Such an encroachment upon the VA could impede venous drainage, leading to increased venous pressure. There is also a report describing a patient with the classic triad of MD: vertigo, tinnitus, and hearing loss as the sole symptom of a radiologically detected thrombosis of

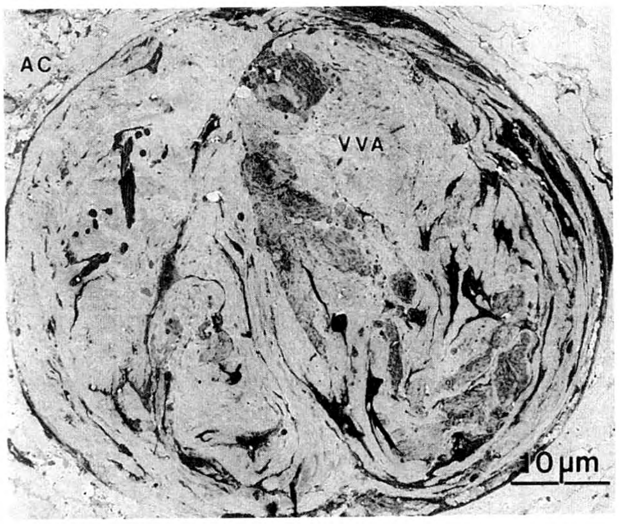


Fig 8. TEM image of same occluded vein of vestibular aqueduct (VVA) as in Fig 7, sectioned 50 µm more distal than in Fig 7 (original ×1,600). Vessel is totally occluded, with no signs of recanalization. AC — accessory canal.

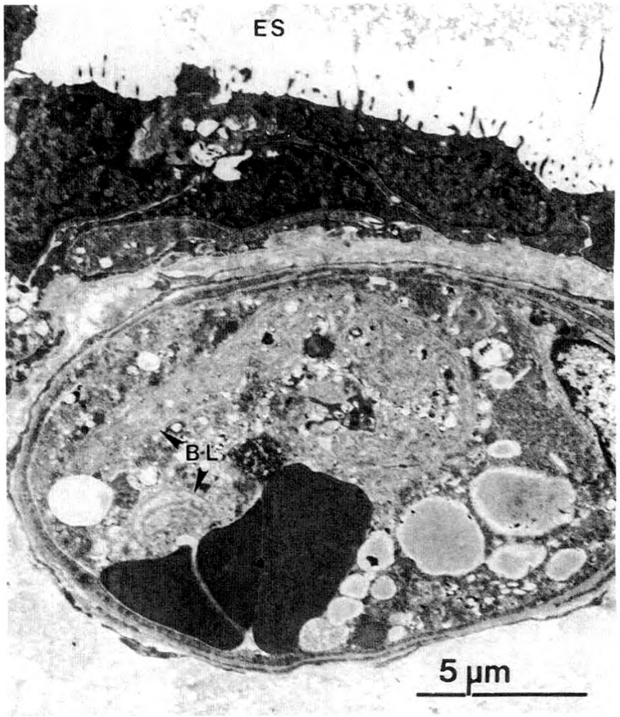


Fig 9. TEM image of subepithelial, partially obliterated vessel (original ×6,650). Occluding material shows amorphous or fibrous material, lipid deposits, and basal lamina (BL) formation (arrowheads). Note layers of concentrically arranged BL (downward-pointing arrowhead). ES — endolymphatic sac.

the lateral sinus and jugular bulb.[23]

In the present study, occlusion of the VVA and partial obliteration of the ES microvasculature were found in 2 MD patients at the level of the proximal ES. The distal end of the VVA occlusion could not be ascertained. This occlusion could lead to impeded venous drainage from the parts mainly involved in endolymph resorption: the ED and proximal ES. This condition resembled the animal model of Lee and Kimura[14] from 1992, in which the venous drainage through the VVA was blocked distally. The findings in the MD patients in the present study — generally flattened epithelial cells, subepithelial fibrosis, and the sparse presence of homogeneous luminal substance in the ES — resembled the findings in ES ischemia.[14] Reduced fluid resorption in the ED and/or ES could result in increased endolymphatic fluid volume and pressure. In an animal model, an increase in endolymphatic pressure was shown to induce enzymatic degradation of the homogeneous substance in the ES.[4] The secretory functions of the ES could also be affected in a condition of decreased microcirculation. Thus, the finding of a reduced amount of homogeneous substance in the ES could be a result of increased enzymatic activity caused by increased endolymphatic pressure, decreased secretory activity, or both. Areas of fibrosis, calcification, and new bone formation in the perisaccular tissue were previously described also in subjects without MD.[24] Comparison with the 7 schwannoma or meningioma patients revealed that subepithelial flattening, subepithelial fibrosis, and signs of new bone formation were more common in the MD patient group (see Table).

The nature of the occluding material in the VVA and ES vasculature is unknown. Earlier ultrastructural analysis of advanced atherosclerotic lesions from the femoral artery in humans showed dense connective tissue with flat, pancake-shaped smooth muscle cells in a lacunalike space.[25] This space consisted of concentric layers of basement membrane, collagen fibrils, and proteoglycan. Some lesions had foci of degeneration, calcification, and lipid deposits.[25] Such lesions, as well as concentrically arranged basal lamina formations, were also found in the vessels of the MD patients. This finding could indicate that the occluding material consisted of thrombi and that the lesions in some cases were advanced and long-standing. The surgical trauma of drilling in performing labyrinthectomy and partial sacectomy could be one causative factor inducing vascular occlusion. However, the VVA and perisaccular vessels of patients with acoustic neuroma or meningioma, subjected to the same surgical trauma, did not show signs of such occlusion (see Table).

All patients in the study had undergone 1 or more therapeutic procedures, all intended to have an ablative effect on the vestibular sensory epithelium (see Table). Patients 1, 2, and 3 had undergone 2 ultrasound operations. The surgical procedure included a simple mastoidectomy and thinning of the bone to outline the vertex of the 3 semicircular canals. Because the ES was not visualized, damage to the VVA with consequent disturbance of its venous drainage was unlikely to occur. In animal experiments, ultrasonic waves directed through the otic capsule produced degenerative changes, not only in sensory and supporting elements, but also in blood vessels and nerve fibers.[26] In the acute phase after irradiation, congestion with sludging of the red blood corpuscles of the capillaries was observed in the connective tissue stroma of the ampullar cristae.[27] The chronic changes included more fibrous connective tissue than normal, with few remaining blood vessels.[26] The way in which ultrasound caused degeneration was earlier thought to be thermal, but mechanical forces are now believed to be more important in inflicting biological damage. In patients 1, 2, and 3, ultrasound was given on 2 separate occasions: in patient 1 with a 2-year interval, in patient 2 with a 7-month interval, and in patient 3 with a 1-year interval. Some 2 to 3 years elapsed between the last ultrasound procedure and labyrinthectomy. The presence of vascular occlusion varied in the 3 patients who underwent this treatment. Only patient 1 had numerous occluded vessels, including the VVA, whereas patients 2 and 3 showed no signs of microvascular occlusion. These patients had also undergone additional transtympanic gentamicin treatment. In the present study, it seemed reasonable to assume that the substantial depth at which the VA is located, separated from the points of irradiation by several millimeters of bone, would protect the periductal and perisaccular vasculature from the thermal or mechanical effects of irradiation.

Three of the 4 patients received gentamicin transtympanically. Patients 2 and 4 had 1 course of 3 daily injections, and patient 3 had 2 courses, with 3 initial injections and 2 more injections after 3 months. Experimental studies on the vitreous body and skin wounds have shown that high local concentrations of gentamicin can stem the regional blood flow partly or completely.[28,29] Several animal studies revealed morphological damage by aminoglycosides administered transtympanically on inner ear tissues. The studies generally used a sustained-release vehicle impregnated with gentamicin to ensure a more certain administration through the round window membrane. Hair cell degeneration with missing or disorganized hair bundles was seen in the cochlear and vestibular sensory cell regions.[30,31] The toxic effects in animal experiments, however, occurred at concentrations considerably in excess of those used clinically in humans. The current dose for clinical transtympanic administration of gentamicin is 0.5 to 1 mL of 30 mg/mL in a buffered solution. Study of the kinetics of gentamicin uptake into the inner ear of experimental animals has shown that the perilymphatic concentration peaks after 12 hours at about 10% of the middle ear concentration with use of a sustained-release vehicle.[32] It was found that the concentration in endolymph reached 20% of that in perilymph.[33] The damage to hair cells was seen to diminish with increasing distance from the septum cruciatum and round window membrane, indicating a gradual weakening of gentamicin concentration.[31] In experimental animals, dark-staining endolymph, cellular debris, and macrophages were noted in the ES lumen after intratympanic application of aminoglycosides.[34] Macrophages and mononuclear cells formed aggregations in the luminal space in the proximal ES in patient 1, who was the only patient who had not received gentamicin.[15] Such findings were not seen in the gentamicin patient group. The distance between the round window membrane and the ED and ES would reduce the concentration of gentamicin to locally harmless levels. Because the gentamicin was not administered in a sustained-released suspension, the duration of action on the round window membrane was short. Hence, damage to the ED and ES structures was not expected from locally administered gentamicin in the present patient group. In this group, the presence of vascular occlusion varied. Patients 2 and 3 showed no signs of perisaccular vessel occlusion, despite having received 1 and 2 courses of treatment, respectively, whereas patient 4 had massive changes in the ED and ES vasculature after 1 course of treatment. The lumen of the VVA in this last patient was patent proximal to the isthmus level of the ED, so the occlusion may have been confined to the proximal ES microvasculature. If gentamicin had generated the occlusion, the entire VVA ought to have been obliterated.

Autoimmune disease is known to be one of the insults that can trigger microvascular thrombosis. Yazawa and Kitahara[35] earlier described microvascular injury caused by immune complex deposition in the ES in patients with MD. In 1 of the patients in the present study (No. 1), there were signs of inflammatory disease in the ES, with mononuclear cells focally invading the epithelial layer, leading to pathological changes such as apoptosis and accumulation of immune complexes subepithelially and at the abluminal aspect of the ES vessels.[15] This patient with "endolymphatic sacitis" showed occlusion of the VVA and also occluded perisaccular and periaqueductal blood vessels. Whether these findings reflect manifestations of an immune-mediated disease cannot be determined from the present data.

A hypercoagulability condition could manifest itself in the development of venous thrombosis. This outcome was seen experimentally in Mongolian gerbil ESs after water deprivation that led to hyperosmosis and changes in the morphology of red blood cells. The condition resulted in thrombus-like occlusion of some of the capillaries.[36] Another factor able to cause hypercoagulability could be hypofibrinolysis, which was found in 5 patients with MD in a Danish study.[12]

The presence of vascular impairment in the ES in MD patients indicates that altered hemodynamics may contribute to the pathogenesis of endolymphatic hydrops and MD. Some of the earlier therapeutic methods used for MD included nicotinic acid or blockage of the stellate ganglion, which were intended to increase the inner ear circulation. The beneficial effects of these treatments were dubious, however, and the treatments have now been abandoned. In the present study, vascular occlusion was found in 2 patients with MD. Whether the present findings will generate any innovations in clinical therapy for this challenging disease remains to be seen. Such treatments might use a transtympanic administration route, which could offer an interesting alternative for local treatment of inner ear microvascular disease.

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MALLEUS FIXATION: CLINICAL AND HISTOPATHOLOGIC FINDINGS

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The goals of this study were to review important clinical and histopathologic features of malleus fixation. Ten clinical cases and 10 histopathologic cases of malleus fixation were identified. For the clinical cases, preoperative clinical data, surgical findings, and preoperative and postoperative audiometric findings were reviewed. Ninety percent of the clinical cases achieved significant reduction of the air-bone gap after operation. Two thirds of these cases had an air-bone gap of 10 dB or less, and the remainder had air-bone gaps between 20 and 28 dB after operation. For the temporal bone cases, clinical data, histopathologic findings, and other otologic diagnoses were reviewed. Malleus fixation can be idiopathic or a result of trauma, chronic otitis media, or developmental anomalies. It is a cause of hearing loss that is likely to be more common than the number of diagnoses would indicate. Certain audiometric findings may lead one to suspect the diagnosis. The surgical approach used depends on the individual anatomy, and surgery is usually highly effective in improving hearing.

KEY WORDS — conductive hearing loss, malleus fixation, otology, otosclerosis.

INTRODUCTION

Malleus fixation is one possible cause of failure of stapedectomy or tympanoplasty with an intact ossicular chain. In 1860, Toynbee reported finding fixation of the malleus or the malleus and incus in 5 ears.[1] In 1967, Guilford and Anson[2] described 30 patients in a series of 3,991 stapedectomies in whom fixation of the head of the malleus to the wall of the epitympanum was identified. That report was one of the first to emphasize the importance of checking the ossicular chain for a fixed malleus in every stapedectomy to avoid the disappointment of persistent conductive hearing loss. Katzke and Plester[3] also described a similar incidence of isolated fixation of the malleus (15 out of 2,542 stapedectomies over a 6-year period).

Clinically, most patients with malleus fixation present in the later decades of life with gradual progressive conductive hearing impairment. The majority of cases of malleus fixation are idiopathic and possibly congenital. Yet, a common clinical presentation includes a gradual progressive hearing impairment. No particular gender differences have been noted. The diagnosis can be made on pneumatic otoscopy by noting the absence of movement of the manubrium, umbo, or lateral process. Evidence of tympanosclerosis of the tympanic membrane raises suspicion, as this is a common cause of malleus fixation.

Audiometry frequently shows an air-bone gap suggestive of conductive hearing loss, but may often show a mixed hearing loss. Katzke and Plester[3] emphasize the importance of the sensorineural component of the hearing loss in malleus head fixation. The cause of this sensorineural component is unknown. Tympanograms, as in otosclerosis, show reduced or normal compliance. It is important to note that the vast majority of cases of malleus fixation are unilateral, whereas otosclerosis is frequently a bilateral lesion.

CASE MATERIAL AND METHODS

The clinical records at the University of California–San Diego School of Medicine and the Massachusetts Eye and Ear Infirmary were searched for the diagnoses of "ossicular fixation" and "ossicular discontinuity." The inclusive dates of the search extended from March 1991 to September 1999. Ten patients with malleus fixation were identified. These patients were then classified by underlying otologic diagnosis. Six of the 10 patients (67%) had idiopathic malleus head fixation with no associated otologic diagnosis. Two cases occurred in the setting of chronic otitis media (COM); 1 of these patients also had tympanosclerosis. One case occurred after trauma, and 1 in a patient with Paget's disease.

In addition, the temporal bone library at the Massachusetts Eye and Ear Infirmary was searched for

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TABLE 1. CLINICAL CASES OF MALLEUS FIXATION

Case	*Age (y)*	*Sex*	*Preoperative Audiogram*	*Postoperative Audiogram*	*Preoperative Diagnosis*	*Underlying Otologic Diagnosis*	*Surgical Findings*	*Surgical Treatment*
1	63	M	R, moderate mixed loss, severe at 8 kHz	R, closure of air-bone gap 250-4,000 Hz	Otosclerosis	None (idiopathic fixation)	R, malleus and incus fixed in epitympanum	Atticotomy with removal of incus and malleus head; malleus head used as autograft
2	44	M	Mild to moderate bilateral mixed loss, L > R	R, thresholds decreased 20-25 dB	Conductive hearing loss	None (idiopathic fixation)	R, malleus fixed in epitympanum superiorly by broad band of fixation	Incus and malleus head removed; malleus head used as autograft
3	40	F	L, mild to moderate conductive loss	L, closure of air-bone gap	Otosclerosis	None (idiopathic fixation)	L, osseous fixation from anterior epitympanum to neck of malleus	Osseous fixation drilled away to free malleus (intact ossicular chain left)
4	9	F	L, moderate conductive loss (30- to 40-dB air-bone gap)	L, air-bone gap decreased to 15 dB	Congenital ossicular fixation with conductive hearing loss	None (idiopathic fixation)	L, fixed malleus head and incus body in epitympanum	Incus and malleus head removed; incus used as interposition graft
5	48	M	L, 30-dB air-bone gap	L, air-bone gap decreased to 15 dB in lower frequencies	Otosclerosis	None (idiopathic fixation)	L, malleus fixed, incus and stapes mobile	Incus and malleus head removed; incus used as interposition graft
6	39	M	Bilateral downsloping sensorineural loss, conductive losses 20 dB on R, 40 dB on L	L, closure of air-bone gap	Otosclerosis	None (idiopathic fixation)	L, incudomalleal complex fixed	Incus and malleus head removed; incus used as interposition graft
7	41	M	R, moderate to severe conductive loss 250-8,000 Hz	Pending	Chronic otitis media with conductive hearing loss	Chronic otitis media	R, malleus fixed in epitympanum laterally	Band of fixation drilled to free malleus (intact ossicular chain left)
8	12	F	R, 50-dB air-bone gap	R, 45-dB air-bone gap	Chronic otitis media	Chronic otitis media, tympanosclerosis	R, fixed malleus and incus with malformed ossicles	Fused malleus head–incus removed and used as interposition graft
9	38	F	L, 60- to 70-dB air-bone gap 250-2,000 Hz; sensorineural loss with 30-dB air-bone gap at higher frequencies	L, closure of air-bone gap with improvement in sensorineural loss as well	Conductive hearing loss caused by trauma	Trauma (motor vehicle accident at age 13 y with subsequent conductive hearing loss)	L, fixed malleus	Incus removed and used as interposition graft
10	75	M	Mixed loss, R > L	R, mild improvement in lower frequencies	Conductive hearing loss caused by Paget's disease	Paget's disease	R, entire ossicular chain (malleus, incus, and stapes) stiff	R, stapedectomy; incus removed; malleus head nipped; malleus-to–oval window fat wire prosthesis inserted

cases of malleus fixation to study the histopathology of this entity. A total of 10 cases of malleus fixation were identified. Of these, 5 had no underlying otologic diagnosis, and hence the cause was presumed to be idiopathic or congenital. One case occurred in a patient with a history of COM, and 1 case

TABLE 2. HISTOPATHOLOGIC CASES OF MALLEUS FIXATION

Case	*Age (y)*	*Sex*	*Audiogram*	*Underlying Otologic Diagnosis*	*Histopathology*	*Surgery or Treatment*
11	63	F	Not available	None (idiopathic fixation)	L, bony bar from head of malleus to anterior epitympanic wall	None
12	89	F	Bilateral sensorineural loss 40-60 dB on R, 60 dB on L	None (idiopathic fixation)	L, osseous fixation of head of malleus to anterior epitympanic wall	None
13	89	M	Bilateral severe sensorineural loss, R > L	None (idiopathic fixation)	R, head of malleus with fibrous fixation to bony spur; L, fibrous fixation of malleus to bony spur in epitympanum	None
14	53	M	Not available	None (idiopathic fixation)	L, fixation of head of malleus to anterior epitympanic wall by bony bar	None
15	66	M	Bilateral sensorineural loss; R, flat 20-dB loss to 1,000 Hz, flat 50- to 60-dB loss for 2,000 to 8,000 Hz; L, low-tone loss below 1,000 Hz, descending loss above 1,000 Hz	None (idiopathic fixation)	L, bony bar fixing malleus to anterior epitympanic wall	None
16	85	F	Bilateral conductive loss approximately 35 dB	Tympanosclerosis	R, tympanosclerosis in epitympanum approximating head of malleus; L, numerous middle ear large hyaline plaques that surround and fix malleus, incus, and fractured stapes	Mobilization of stapes at age 53 y failed to improve hearing
17	67	F	Bilateral conductive loss, L > R	Chronic otitis media	R, head of malleus fixed to superior epitympanic wall by small bar (likely osteogenesis caused by infection)	None
18	90	F	R, profound hearing loss; L, moderate sensorineural loss and mild conductive loss	Paget's disease, R otosclerosis, (also history of mumps in childhood)	L, malleus fixed to anterior epitympanic wall by spur of bone	None
19	60	F	Not available (Rinne negative both sides, Weber to R)	Otosclerosis	L, ankylosis of malleus to anterior epitympanic wall by bony bar	None
20	81	F	Preoperatively, bilateral mixed loss with flat thresholds; postoperatively, closure of air-bone gap on L, 25-dB air-bone gap persists on R	Otosclerosis	R, head of malleus fixed to anterolateral wall of epitympanum by solid bar of lamellar bone	Age 69 y, L stapedectomy (successful); age 70 y, R stapedectomy (residual air-bone gap)

occurred in the setting of tympanosclerosis. Two cases were found in patients with documented otosclerosis affecting the stapes footplate. Interestingly, 1 case of malleus fixation was found in a patient with underlying Paget's disease that did not involve the temporal bone. The clinical features, as well as the histopathologic findings of these cases, are presented.

RESULTS

Idiopathic or Congenital Malleus Head Fixation. A total of 6 clinical cases and 5 histopathologic cases (from the temporal bone search) of idiopathic malleus fixation were identified (Tables 1 and 2). The preoperative diagnoses in the clinical cases included otosclerosis in 4 cases (these patients were subsequently not found to have otosclerosis at the time of surgery), conductive hearing loss in 1 case, and congenital ossicular fixation with conductive hearing loss in 1 case. Three of the 6 cases had fixation of both the malleus and the incus within the epitympanum. The remaining 3 cases had fixation of the malleus, but normal mobility of the incus and stapes. Three cases were corrected by use of the incus as an interposition graft, 2 cases were corrected by use of the malleus head as an autograft, and in 1 case, in which the malleus was fixed in the epitympanum laterally, the focus of fixation was drilled away to free the malleus. The preoperative and postoperative audiograms for the last case are illustrated in Fig 1. Closure of the air-bone gap to within 10 dB was achieved.

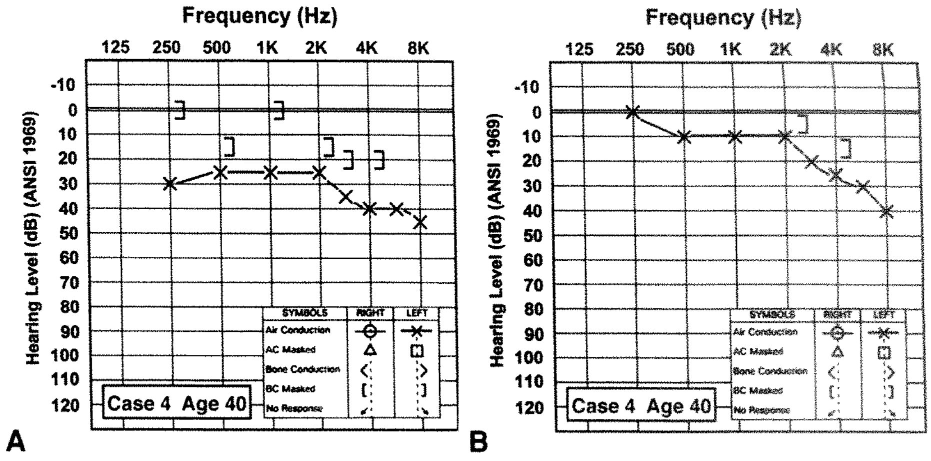

Fig 1. (Case 4) **A)** Preoperative and **B)** postoperative audiograms for case of idiopathic malleus head fixation.

The histopathologic findings of idiopathic malleus head fixation confirm that there are various possible sites of fixation within the epitympanum (Table 2). The fixation in the 5 cases identified from the temporal bone library varied from bony ankylosis of the malleus head to the epitympanic wall to fibrous fixation of the malleus to a bony spur (Fig 2).

The audiological findings in idiopathic malleus fixation are presented in Table 3. All of the cases demonstrated a significant air-bone gap with normal or flat tympanograms before operation. In the 3 cases in which acoustic reflexes were tested before operation, the reflexes were absent in the affected ear. The air-bone gap typically narrowed at the higher frequencies (2 to 8 kHz). This trend is seen in all of the cases of malleus fixation reviewed here and is consistent with a stiffness-type lesion.

Chronic Otitis Media. Two clinical cases and 1 histopathologic case of fixation of the malleus head in the setting of COM were identified (Tables 1 and 2). The first case occurred in a patient with a history of COM and conductive hearing loss on the right side. The preoperative audiogram (Fig 3) showed a moderate to severe conductive hearing loss on the right. Exploratory tympanotomy was performed on the right side for possible otosclerosis. However, there was no focus of otosclerosis noted during the operation. The malleus was noted to be fixed to the lateral epitympanum. The focus of fixation attaching the malleus to the epitympanum was drilled away. After operation, the patient's hearing was significantly improved. However, the patient was lost to follow-up before a postoperative audiogram could be obtained.

In the second case of malleus fixation with underlying COM, both the malleus and incus were fixed. This case was corrected by use of the fused malleus head–incus as an interposition graft between the stapes and the tympanic membrane.

The histopathologic case of malleus fixation in the setting of COM revealed a malleus head fixed to the superior epitympanic wall by a small bar that was

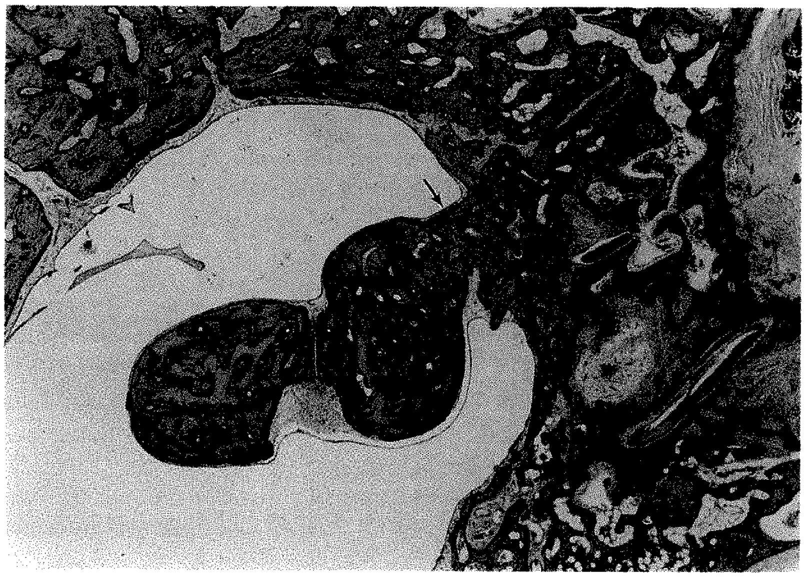

Fig 2. (Case 12) Osseous fixation (arrow) of head of malleus to anterior epitympanic wall (left ear).

TABLE 3. AUDIOLOGICAL FINDINGS IN PATIENTS WITH MALLEUS FIXATION

		*Air-Bone Gap (dB)**						
Case	*Ear*	*<500 Hz*	*500 Hz*	*1,000 Hz*	*2,000 Hz*	*4,000 Hz*	*Tympanogram*	*Reflexes*
1	R	50/20	40/10	25/20	30/0	10/0	Normal/NA	Absent bilaterally/NA
2	R	25/15	25/5	15/15	15/10	0/0	Flat on R/NA	Absent bilaterally/NA
3	L	30/0	30/0	30/0	40/0	10/10	Type A/NA	NA/NA
4	L	50/20	50/30	30/25	15/10	15/15	NA/NA	NA/NA
5	L	30/20	10/20	25/10	10/10	20/0	Normal bilaterally/NA	Absent on L, normal on R/NA
6	L	40/15	30/5	35/0	10/0	30/0	NA/NA	NA/NA
7	R	45/NA	45/NA	45/NA	20/NA	5/NA	Normal bilaterally/NA	NA/NA
8	R	50/60	55/60	50/45	30/30	30/40	Tympanic membrane perforation consistent with middle ear effusion bilaterally	NA/NA
9	L	75/10	80/5	70/10	45/10	15/0	NA/NA	NA/NA
10	R	70/30	50/15	30/15	15/25	30/55	Flat on R/NA	Absent bilaterally/NA

Data are preoperative/postoperative.

NA — not available.

*Bone threshold minus air threshold.

likely to have been neo-osteogenesis caused by infection (Fig 4).

The audiological findings in cases of fixation of the malleus in COM are similar to those of idiopathic malleus head fixation, with air-bone gaps narrowing at the higher frequencies.

Trauma. One clinical case of malleus fixation occurring after trauma was identified (Table 1). The patient was a 38-year-old woman with a history of a motor vehicle accident at 13 years of age with subsequent left conductive hearing loss. The preoperative audiogram showed a very large air-bone gap (65 dB) from 250 to 2,000 Hz and a 30-dB air bone gap at the higher frequencies (Fig 5A). The patient was taken to the operating room for exploratory tympanotomy. Palpation of the malleus and the incus during surgery revealed fixation. The incus was fashioned into an interposition graft between the stapes and the tympanic membrane. The resulting mobilized malleus was left in place. The postoperative audiogram showed closure of the air-bone gap at all frequencies (Fig 5B). No histopathologic examples of malleus fixation caused by trauma were identified.

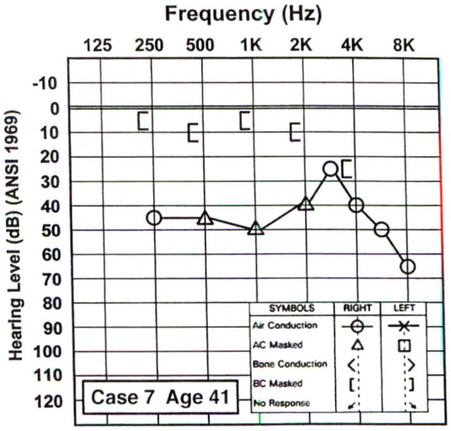

Fig 3. (Case 7) Preoperative audiogram for case of malleus fixation caused by chronic otitis media.

Paget's Disease. One clinical case of malleus fixation occurred in a patient with a long-standing history of Paget's disease of bone (Table 1). The preoperative audiogram showed a mixed hearing loss with a significant air-bone gap, worse on the right side (Fig 6A). A tympanogram showed a flat tracing, and the acoustic reflexes were absent bilaterally. A temporal bone computed tomography (CT) scan showed extensive involvement of the otic capsule by Paget's disease. No narrowing of the internal auditory canal or epitympanum was noted. It was decided to proceed with an exploratory tympanotomy on the right ear to further evaluate the cause of the hearing loss. The malleus and incus were found to be quite stiff, as was the stapes. The oval window niche was narrowed, presumably by pagetoid changes of the promontory. The stapes was removed in its entirety. The incus and the head of the malleus were removed, and a malleus-to–oval window fat wire prosthesis was inserted. The postoperative audiogram, however, failed to show significant hearing improvement, although the patient gained 10 to 15 dB of improvement in the low frequencies (Fig 6B).

The temporal bone search revealed 1 case of a patient with Paget's disease who was also noted to have concomitant malleus fixation on the left (Table 2). This patient also had otosclerosis in the right ear. An audiogram showed a profound hearing loss in the right ear; the left ear showed a moderate sensorineu-

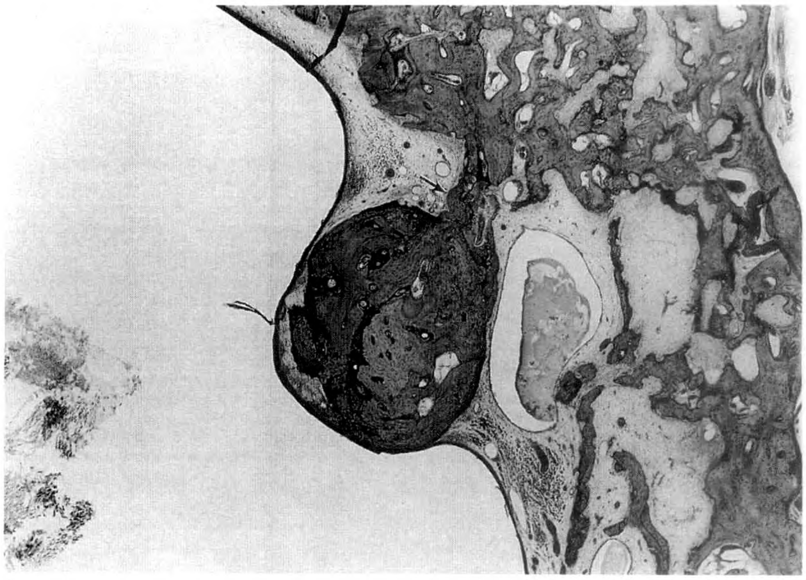

Fig 4. (Case 17) Head of malleus is fixed to superior epitympanic wall by small bar (arrow), likely because of osteogenesis caused by chronic otitis media.

ral hearing loss with a superimposed mild conductive loss. This patient was also noted to have a history of mumps in childhood. Careful histopathologic examination of the temporal bone, however, showed no convincing evidence of involvement of the temporal bone by Paget's disease. The left ear was, however, noted to have malleus fixation to a spur of bone from the anterior epitympanic wall (Fig 7). Presumably, this was an incidental malleus fixation in a patient with Paget's disease.

Otosclerosis. Two cases of malleus fixation in patients with histopathologically confirmed otosclerosis were identified in the temporal bone search (Table 2). The first case (case 19) had a conductive hearing loss present bilaterally as evidenced by negative Rinne tests bilaterally. No audiograms were available. Histopathology showed ankylosis of the malleus to the anterior epitympanic wall by a bony bar on the left, with no evidence of otosclerotic bone in this region, but with otosclerotic fixation of the stapes. The left ear also showed otosclerotic foci, but not in the region of the malleus.

The second case (case 20) had a preoperative audiogram available; it showed mixed hearing losses on both sides. The patient underwent a stapedectomy in the left ear at 69 years of age, and one in the right ear at 70 years. After the operations, a 25-dB air-bone gap persisted in the right ear, and the left ear air-bone gap was closed (Fig 8A). Histopathology (Fig 8B) demonstrated that the malleus head was fixed to the anterolateral wall of the epitympanum by a solid bar of lamellar bone. This case illustrates the importance of identifying malleus fixation at the time of stapedectomy, as it can be a cause of persistent conductive hearing loss in an apparently successful stapedectomy.

DISCUSSION

Sites of Fixation. The site of fixation of the malleus in either idiopathic or secondary malleus fixation is important, as it often determines the surgical approach used to correct the defect. A review of the literature reveals that in the majority of cases, malleus fixation occurs in the epitympanum. In a histologic

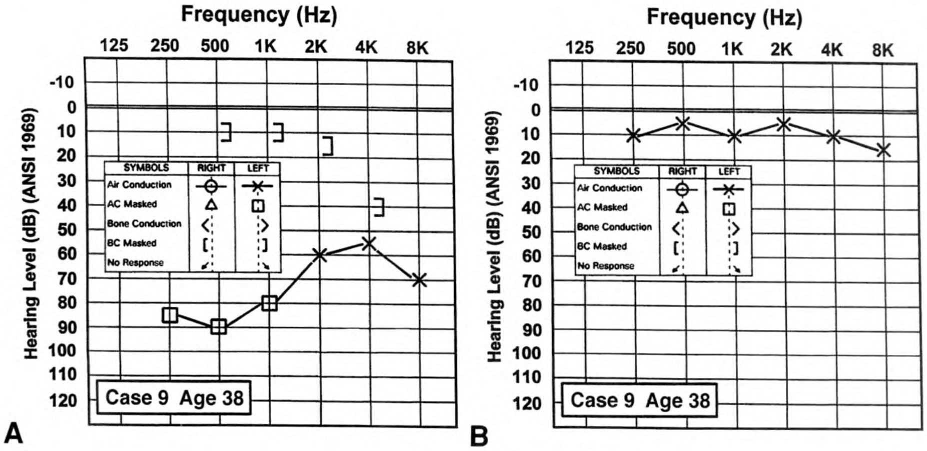

Fig 5. (Case 9) **A)** Preoperative and **B)** postoperative audiograms for case of malleus fixation caused by trauma.

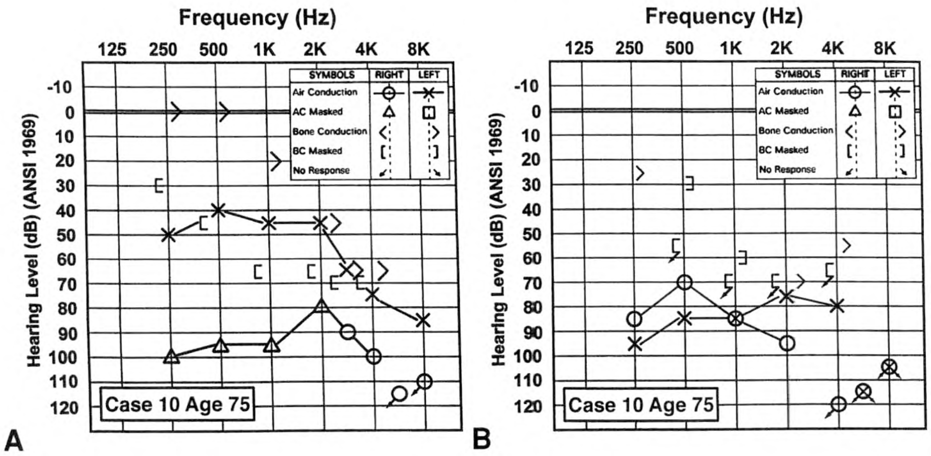

Fig 6. (Case 10) **A)** Preoperative and **B)** postoperative audiograms for case of bilateral conductive hearing loss caused by Paget's disease. **A)** Right ear is worse than left. **B)** Postoperative audiogram shows no significant improvement in hearing on right.

analysis of 1,108 temporal bones by Subotic et al,[4] 14 cases of bony fixation of the malleus were found. The fixation was noted to be almost always unilateral, and the most frequent site of fixation was the lateral epitympanic wall. A second group had fixation at the epitympanic roof. The forms of fixation varied from a thin bony lamella to a solid bony bridge.[4] Moon and Hahn[1] and Tos[5] have reported fixation due to ossification of the anterior or superior ligaments of the malleus. Congenital fixation of the malleus can also occur by incomplete differentiation of the sphenomandibular ligament resulting in a bony spicule from the anterior malleus head to the anterior tympanic fissure.[6] Ossification of the tendon of the tensor tympani can also simulate fixation of the head of the malleus. Finally, in acquired cases, the malleus head can be fixed to the anterior, lateral, superior, or medial wall of the epitympanum.[3]

Etiology and Pathogenesis. A number of theories have been proposed for the pathogenesis of malleus fixation. These include tympanosclerosis, trauma, chronic infection of the ear, otosclerosis, and even developmental anomalies of the tympanic cavity. A rare cause of malleus fixation can also be Paget's disease of the temporal bone.[7] Tympanosclerosis is the most common cause of malleus fixation, accounting for 43.5% of cases in the series of Katzke and Plester.[3] Idiopathic malleus fixation was, however, noted to be the second most common cause. Tympanosclerosis and idiopathic malleus fixation accounted for more than 80% of cases; the remainder were accounted for by trauma, COM, otosclerosis, or developmental anomalies.

It is easy to see how a tympanosclerotic mass may lead to fixation of the malleus or even of the entire ossicular chain in advanced cases. The cause, extent, and site of the lesion can be fully evaluated on surgical exploration. Chronic otitis media, as mentioned above, can also lead to fixation of the malleus. As proposed by Tos,[5] postinfectious degeneration of the ligaments followed by calcification and ossification can result in bony fixation of one or more ossicles. The chronic inflammation induced by a cholesteatoma may also lead to neo-osteogenesis in the walls of the epitympanum.

Trauma leading to fracture of the temporal bone

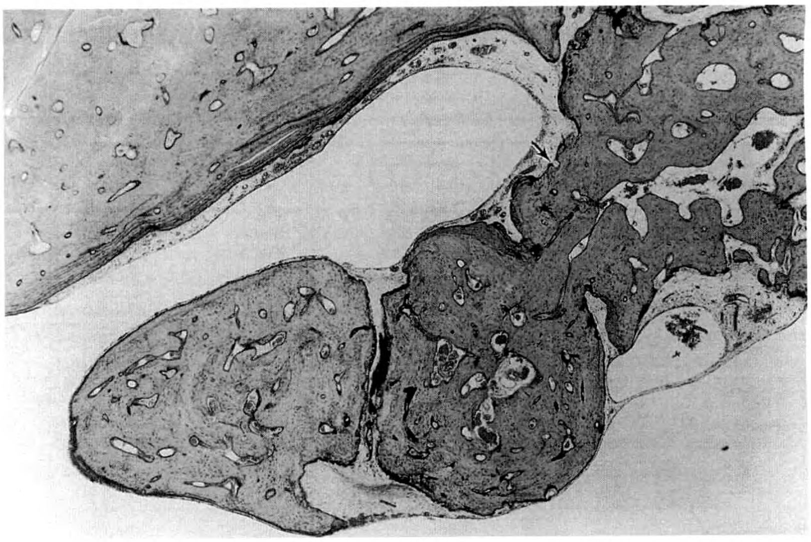

Fig 7. (Case 18) Malleus is fixed to anterior epitympanic wall by spur of bone (arrow); patient has Paget's disease.

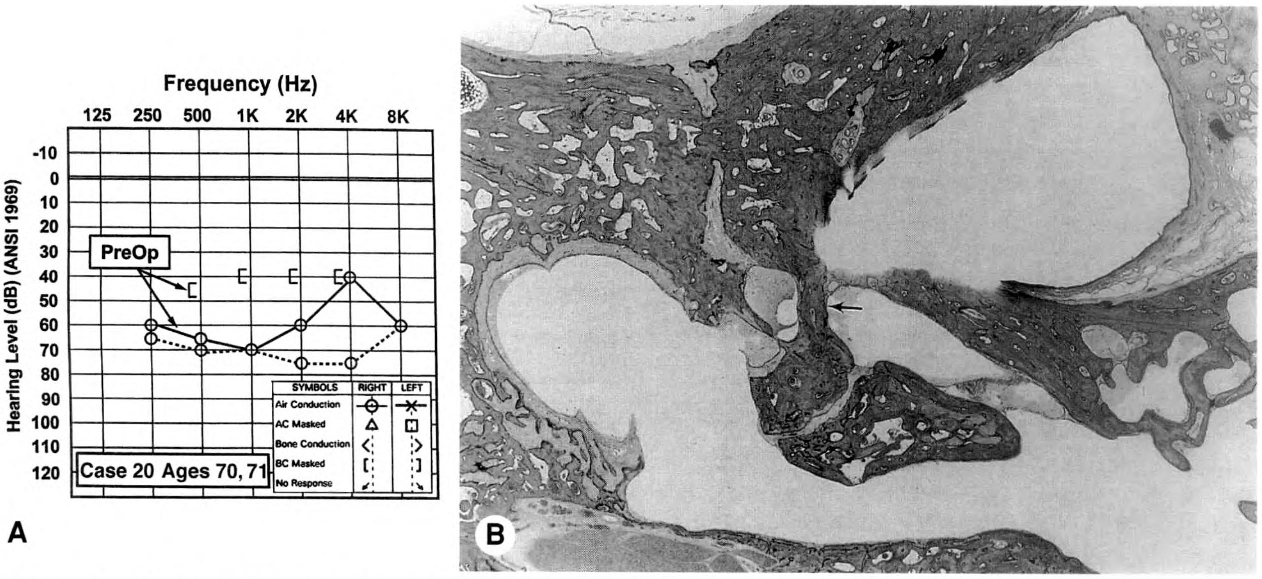

Fig 8. (Case 20) **A)** Solid line indicates preoperative thresholds, dotted line indicates post-stapedectomy thresholds. As can be seen, 25-dB air-bone gap persists, presumably because patient had malleus fixation on right. **B)** Head of malleus is fixed to anterolateral wall of epitympanum by bar of lamellar bone (arrow).

may fix the malleus as callus formation occurs. Surgical trauma may also predispose to malleus fixation by leaving small pieces of bone or dust from drilling, which serve as sites of unwanted bone formation.

Several authors have theorized that an abnormally superior position of the malleus head also increases the risk of bony fixation in the epitympanum. In 1960, Dietzel[8] noted an abnormally superior position of the malleus head in 18% of 55 temporal bones of patients with otosclerosis of the stapes. Professor Ugo Fisch of Zurich, Switzerland, believes that as many as 30% of otosclerosis cases have malleus fixation (personal communication). This tendency is likely due to a shift of the axis of the ossicular chain due to otosclerotic displacement of the footplate.[3] Histopathologic studies of the temporal bone had not previously shown an actual otosclerotic focus to ever be responsible for fixation of the malleus. However, Vincent et al[9] report 2 cases of otosclerosis — 1 of the malleus handle and 1 of the malleus head — causing fixation of the malleus. Vincent et al also compared audiological findings in cases of malleus fixation with those in cases of otosclerosis. Preoperative sensorineural hearing loss was seen more commonly in the malleus ankylosis group. Additionally, in patients with unilateral conductive hearing loss, an important difference was noted between the malleus ankylosis group and the otosclerosis group. The acoustic reflex was absent on the impaired side in all cases, as confirmed by our study; however, the contralateral acoustic reflex was present in 65% of malleus ankylosis patients and *absent* in 70% of the otosclerosis group.[9]

Any developmental anomaly of the epitympanum that brings the malleus in close contact with the walls of the epitympanic cavity is also likely to predispose to malleus fixation. Bony spurs in this region, whether they are developmental anomalies or due to postinflammatory new bone formation, are also likely to increase the risk of malleus fixation. Additionally, since chronic serous otitis media results in medial displacement of the manubrium accompanying retraction of the pars tensa, it would also result in a more lateral position of the malleus head in the epitympanum. Tympanosclerosis is also a well-recognized sequela of chronic serous otitis media. This combination of events may explain the propensity for malleus head fixation to be associated with tympanosclerosis.

Paget's disease (osteitis deformans) is a chronic progressive bony dysplasia. The skull and temporal bones have been noted to be involved in about two thirds of patients.[7] Paget's disease of the temporal bones is associated with conductive deafness, sensorineural deafness, and vestibular dysfunction. Proops et al[10] provide an excellent discussion of the possible causes of the conductive hearing loss in Paget's disease. These include narrowing of the external auditory meatus, thickened tympanic membranes, new bone formation on the medial and lateral walls of the epitympanic recess with resultant fibrous fixation of the ossicles, and stapedial fixation.[10] It is also worthwhile to consider the possibility of a *pseudo*-conductive hearing loss caused by abnormal conduction of sound through a pagetic skull. Although the case presented above fails to definitively demonstrate malleus fixation in pagetic bone, malleus fixation is theoretically one possible cause of the conductive hearing loss that is often seen in Paget's disease involving the temporal bone. However, according to the current literature, as well as the clinical case presented above, surgery performed to correct ossicular fixation associated with Paget's disease often does not improve functional hearing. Thus, surgery cannot be universally recommended as a treatment for conductive hearing loss caused by Paget's disease.

Surgical Treatment. The object of surgery is clearly to liberate the malleus and leave a functional ossicular chain. On any exploration of the middle ear cavity for conductive hearing loss, palpation of the malleus to check for fixation should be a routine practice. It is also important to check the mobility of the stapes initially, as surgical decision-making is based on the presence or absence of stapes mobility. If the malleus head is found to be fixed, the epitympanum should be explored. The early management of malleus fixation involved universal amputation of the malleus head and removal of the incus, which necessitated ossicular reconstruction. Armstrong[11] reported on correction of epitympanic malleus fixation without disruption of the ossicular chain. Creation of an adequate space of about 2 mm around the head of the malleus gives more predictable results than reconstruction of a disrupted ossicular chain.[11] This can be accomplished by widening the attic space or removing as much as two thirds of the anterosuperior aspect of the malleus head. As this may not be possible to do, it may be necessary to remove the head of the malleus and perform an interposition with an incus assembly (transposition of incus between handle of malleus and stapes) in cases in which the malleus head is fixed to the medial epitympanic wall, as it is very difficult to create an adequate space in this situation. Alternatively, the malleus head can be used as an autograft with similar results.

In the case of concomitant stapes fixation, a routine stapedectomy can also be carried out with a wire prosthesis or a Teflon piston.

It is important to exercise great care when drilling around or on the fixed ossicular chain. Tiny round diamond burs should be used at a slow rate of revolution. If the stapes is mobile, it may be advisable to divide the incudostapedial joint first to prevent damage to hearing. It is also important to wash out all bone dust created by drilling, so that it does not cause osteogenesis at unwanted sites.

CONCLUSIONS

Malleus fixation is an important cause of conductive hearing loss that is likely to be much more common than the number of diagnoses would indicate. The reported prevalence in series of stapedectomy patients (3,991 and 2,542 procedures) is 1% to 2%.[2,3] Palpation of the malleus to assess mobility should be routine practice during tympanoplasty and stapedectomy to ensure that this diagnosis is not missed. The diagnosis can often be made before operation on pneumatic otoscopy. A careful history should be taken to elicit any underlying secondary cause. The technique of surgical correction is dictated by the individual anatomy (including mobility of the stapes). In general, however, preservation of the continuity of the ossicular chain gives better results than the reconstruction of a disrupted ossicular chain, although the latter is often necessary because of the individual anatomy.[11]

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ESTIMATED LOCATIONS OF THE NARROWEST PORTION OF THE EUSTACHIAN TUBE LUMEN DURING CLOSED AND OPEN STATES

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To morphologically evaluate the location of the narrowest portion of the eustachian tube (ET) lumen during closed and open states, we used computer-assisted 3-dimensional reconstruction methods to investigate 13 normal human temporal bone–ET specimens obtained from 13 individuals 3 months to 81 years old. On the basis of recent endoscopic observations, we assumed that the site of minimum circumference of the ET lumen represents the site of minimum cross-sectional area during the open state. We tested the hypothesis that the location of the narrowest site of the ET lumen in the closed state is not identical to the site of minimum circumference of the ET lumen. Confirmation of this hypothesis would imply that the site of greatest constriction shifts with contraction of the tensor veli palatini muscle. In 11 of 13 cases, both the site of minimum cross-sectional area and the site of minimum circumference of the ET lumen were located in the cartilaginous portion of the ET, near the anterior margin of the junctional portion. In the 2 remaining cases, the site of minimum circumference was located in the junctional portion of the ET, whereas the site with the narrowest cross-sectional area stayed in the cartilaginous portion. The site of minimum circumference shifted significantly in the direction of the tympanic orifice of the ET, relative to the site of minimum cross-sectional area ($p < .01$). The average distance between these sites was 2.01 ± 1.75 mm. There was no significant difference between children ($n = 6$) and adults ($n = 7$). The so-called isthmus portion of the ET, located between the cartilaginous and bony portions, has been considered traditionally as the narrowest site through the entire ET. However, our results suggest that the narrowest portion of the ET lumen is located in the cartilaginous portion in both closed and open states.

KEY WORDS — computer-assisted three-dimensional reconstruction, eustachian tube function, eustachian tube lumen, isthmus portion of eustachian tube.

INTRODUCTION

The eustachian tube (ET) has 3 major functions: ventilation, clearance, and protection of the middle ear.[1] Constriction of the ET lumen is potentially advantageous for protection of the middle ear from bacteria in the nasopharynx. However, constriction may be disadvantageous for ventilation and clearance of the middle ear. The location and extent of constriction of the ET may have an important impact on middle ear function.

The ET lumen is usually considered to be "closed" in its static state.[2] The ET lumen is known to dynamically open by contraction of the tensor veli palatini muscle (TVPM).[3,4] At the same time, the ET opening may increase clearance of the middle ear and decrease protection of the middle ear. Therefore, the locations of the narrowest portion of the ET lumen may differ when the ET is closed and opened. In our previous study with computer-aided 3-dimensional (3-D) reconstruction and measurement,[5] the narrowest portion of the ET in the closed state was defined as the site in which the lumen has minimal cross-sectional area. This site was located in the cartilaginous portion of the ET. However, little is known about the location of the narrowest portion of the ET lumen when the ET dynamically opens by contraction of the TVPM.

Video endoscopic observations have demonstrated that the shape of the lumen in the isthmus region changes markedly as the ET opens. Poe et al[6] reported that the lumen opens by a dilation of the lateral wall of the ET, which changes the profile of the interior of the lumen from a crescent shape to a round shape. These observations are the consequence of contraction of the TVPM, which moves the lateral membranous wall of the ET[7] and the lateral lamina of the ET cartilage[1] inferolaterally to shift the concave (C-shaped) ET lumen to a round profile. These observations imply that the circumference of the ET lumen is a predictor of the potential cross-sectional area of the dilated ET lumen in the open state.

The purpose of this study was to test the hypoth-

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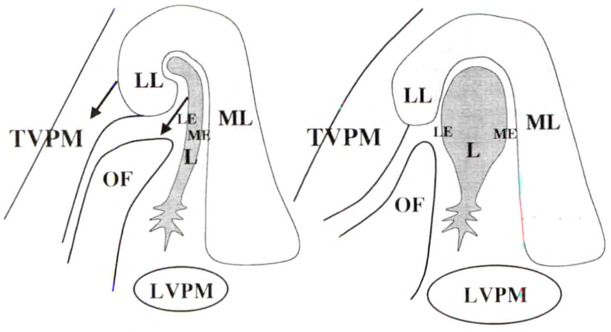

Closed state **Open state**

Fig 1. Schematic drawing of cross section through posterior half of cartilaginous portion of eustachian tube (ET) in closed state and open state. Arrows indicate direction of movement of lateral lamina (LL) of ET cartilage and lateral epithelial lining (LE) of ET away from medial lamina (ML) of ET cartilage and medial epithelial lining (ME) of ET, respectively, when tensor veli palatini muscle (TVPM) contracts. By contraction of TVPM, ET lumen (L), especially its upper portion, dilates inferolaterally. Ostmann's fatty tissue (OF) is located between lower half of L medially and TVPM laterally. Levator veli palatini muscle (LVPM) also seems to assist ET opening in this region.

esis that the site with minimum cross-sectional area and the site with minimum circumference of the ET lumen differ significantly in histologically sectioned human temporal bone–ET specimens. Confirmation of this hypothesis would suggest that opening of the ET is accompanied by a shift of the narrowest portion of the lumen between these sites.

MATERIALS AND METHODS

The materials used for this study were 13 temporal bone–ET specimens obtained from 13 individuals. Their ages were 3, 8, and 16 months and 4, 9, 10, 17, 28, 38, 70, 70, 71, and 88 years at death. None of the individuals had any known congenital anomaly or clinical history or histologic evidence of ear disease. These individuals were divided into 2 groups: children (6 cases, ranging from 3 months to 10 years old) and adults (7 cases, ranging from 17 to 88 years old). The specimens were fixed in 10% formalin, decalcified in 5% trichloroacetic acid, dehydrated in graded concentrations of ethanol, and embedded in celloidin. After reference marks had been made in the celloidin blocks for 3-D reconstruction, the blocks were sectioned at 30 μm in thickness as close to perpendicular to the axis of the ET as possible. Every 30th section was stained with hematoxylin and eosin and prepared for a light microscopic study.[8] Additional section slides between every 30th section were made to determine the exact location of the cartilaginous, junctional,[5] and bony portions of the ET. The junctional portion, located between the cartilaginous and bony portions, was surrounded partly by bone and partly by cartilage.[5] These histologic slides were then used for a computer-aided 3-D reconstruction. The equipment used for reconstruction consisted of a personal computer (NEC PC, 9801RL), a frame buffer (Sapience HyperFrame +) for full-color images, a digitizer (Hitachi HDG-1515, resolution 0.1 mm), and a printer. With the software developed by Takagi and Sando,[9] modified by Fujita and Sando[10] and revised by Sudo et al,[5] accurate reconstruction and measurements could be made of the ET in 3-D fashion.

The ET lumen, the ET cartilage, and the temporal bone bordering the ET were reconstructed for each specimen. After the pharyngeal orifice was defined as the most anterior section containing a complete cross section of the mucosal lining of the ET, the tympanic orifice was defined similarly as the most posterior ET section in proximity to the anterior-superior margin of the tympanic annulus. The center of each orifice was defined as the midpoint of a line bisecting and perpendicular to the line connecting the superior and inferior margins of the ET lumen. This pharyngeal orifice–tympanic orifice line provides a standard long axis for creating a series of approximately 200 virtual cross sections of the ET lumen perpendicular to the axis through the reconstructed images. In these virtual cross sections, both the pharyngeal and tympanic orifices were defined again by the same method mentioned above. We defined the line between the centers of the pharyngeal and the tympanic orifices as the pharyngeal orifice–tympanic orifice distance of the ET. The pharyngeal margin of the junctional portion was defined as the most anterior site in which bone surrounded the ET lumen. Similarly, the tympanic margin of the junctional portion was defined as the most posterior site in which cartilage of the ET was present. The reconstruction methods are described in detail in previous reports.[5,9]

Two assumptions of this study were derived from the results of endoscopic observation of movements of the ET lumen. First, the ET lumen has a characteristic concave or C-shaped profile in vertically cut histologic sections[1] that closely resembles the crescent-shaped lumen profile that was observed by Poe et al[6] in the resting state. Therefore, we have assumed that the crescent shape of the lumen of histologically sectioned ET specimens represents the closed state (ie, the ET dilators, including the TVPM, are relaxed). Second, endoscopic observations[6] indicate that the ET lumen is opened by a dilation of its lateral wall that propagates from the pharyngeal orifice to the isthmus. Because this action produces a round profile of the open lumen in the isthmus region,[6] we have assumed that the site of minimum circumference provides an estimate of the potential site of minimum

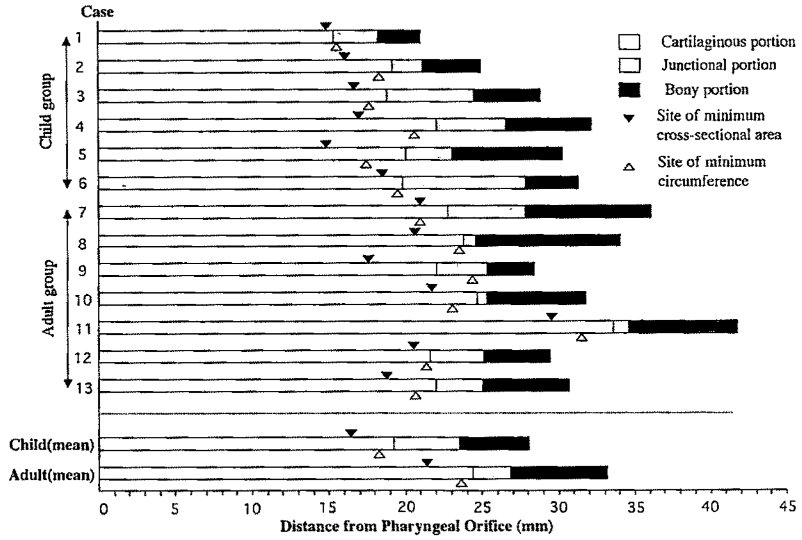

Fig 2. Sites of minimum cross-sectional area and of minimum circumference of ET lumen in each case. Each mean value in both children and adults is also indicated. Note that length of cartilaginous portion of ET is significantly longer in adults than in children (Student's *t*-test; $t = 2.70$, $p < .05$), whereas there is no significant difference in length of junctional and bony portions of ET between children and adults.

cross-sectional area (narrowest portion) of the ET lumen in the open state. Figure 1 illustrates schematic drawings of potential cross sections through the cartilaginous portion of the ET during both closed and open states.

RESULTS

Figure 2 shows the locations of sites of minimum cross-sectional area and minimum circumference of the ET lumen in each case. Statistical analysis revealed that the site of minimum circumference was shifted significantly in the direction of the tympanic orifice of the ET relative to the site of minimum cross-sectional area (paired *t*-test; $t = 4.19$, $p < .01$). In only 1 adult case (case 7) were these 2 sites virtually identical. The average (±SD) distance between these sites was 2.01 ± 1.75 mm (children, 1.81 ± 1.17 mm; adults, 2.19 ± 2.22 mm). There was no statistically significant difference between children and adults. The mean value of the minimum cross-sectional area was 0.62 ± 0.20 mm^2 (children, 0.56 ± 0.23 mm^2; adults, 0.67 ± 0.18 mm^2). The mean value of the minimum circumference was 5.27 ± 0.96 mm (children, 5.21 ± 1.13 mm; adults, 5.32 ± 0.88 mm). There were no statistically significant differences between children and adults. The sites of minimum cross-sectional area were always located in the cartilaginous portion of the ET. However, the sites of minimum circumference in 11 of 13 cases (5 of the 6 children; 6 of the 7 adults) were located in the cartilaginous portion of the ET, adjacent to the pharyngeal margin of the junctional portion of the ET. In the remaining 2 cases (cases 1 and 9), the sites of minimum circumference were located in the junctional portion of the ET. Figure 3 shows photomicrographs of histologic sections of the left ET (case 4) at 4 different sites (the mid-cartilaginous portion, the site of minimum cross-sectional area, the site of minimum circumference, and the junctional portion, respectively, from the pharyngeal side to the tympanic side). The Table summarizes the results of this study.

DISCUSSION

This study demonstrated that the site of minimum circumference of the ET lumen is almost always displaced toward the tympanic orifice of the ET, relative to the site of minimum cross-sectional area. This tendency was found in both children and adults. Hence, we speculate that the location of the narrowest portion of the ET lumen may shift in the direction of the tympanic orifice of the ET when the ET is opened actively. Since contraction of the TVPM is expected to dilate the lumen of the cartilaginous portion of the ET, the narrowest portion in the open state is expected to lie near the site of minimum circumference, which is located in the cartilaginous portion, adjacent to the pharyngeal margin of the junctional portion of the ET. Significantly, the narrowest portion of the ET lumen remains located in the cartilaginous portion during both closed and open states.

The so-called "isthmus portion" of the ET, located between the cartilaginous and bony portions, traditionally has been considered the narrowest site through the entire ET.[11] However, the exact site of the isthmus portion has not been made clear yet. Our results indicate that the pharyngeal margin of the junctional portion of the ET should be designated as the isthmus portion, because the narrowest portion of the ET lumen in the open state (the site of minimum circumference) lies within 1 mm of that site.

The site of minimum cross-sectional area of the ET lumen is expected to be a limiting site for the degree of ET stenosis in the closed state. However, since the anterior margin of the junctional portion of

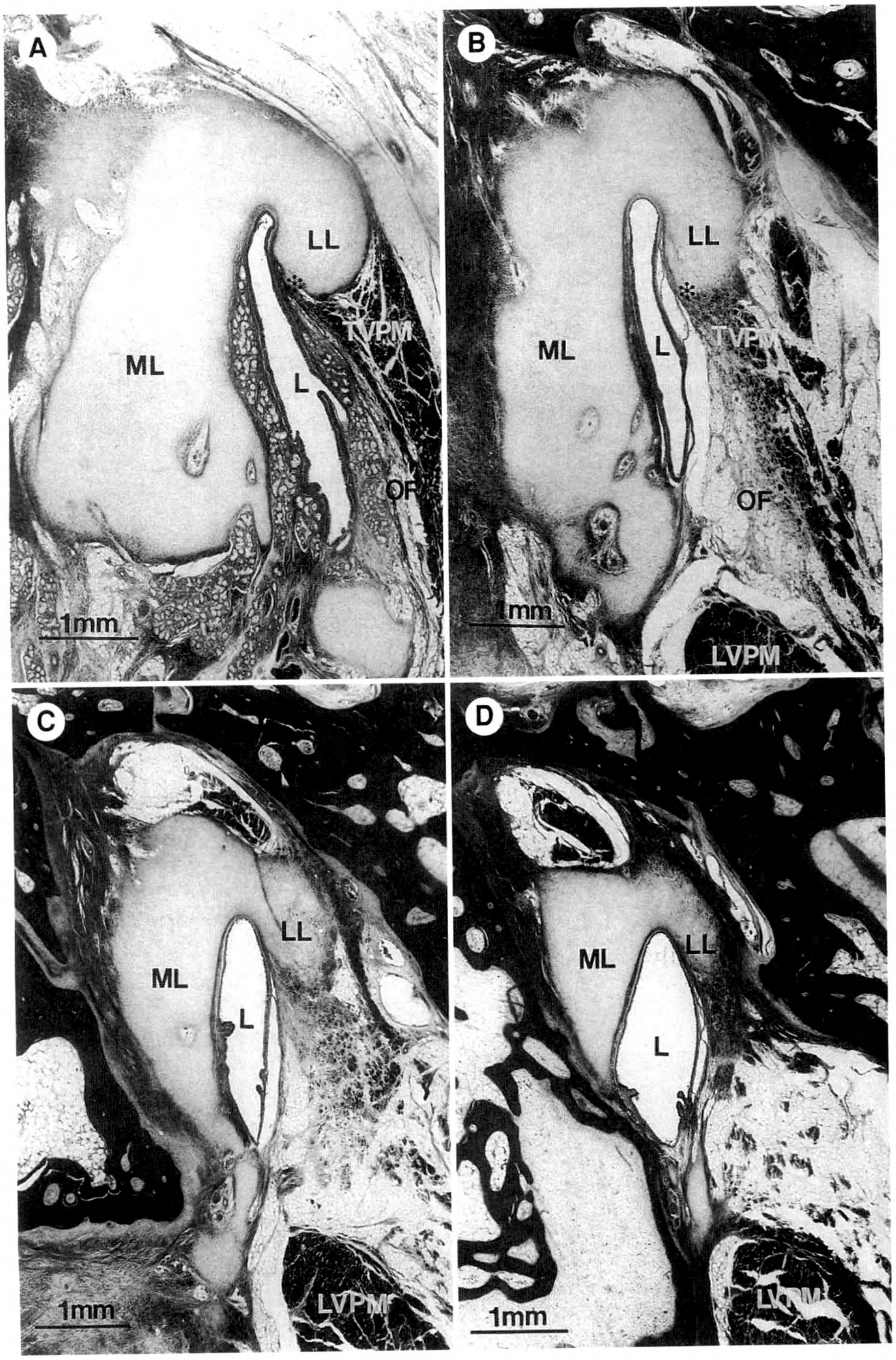

Fig 3. Photomicrographs (anterior view) of vertical sections of left ET and its associated structures obtained from case 4 (4-year-old boy) at 4 different sites (H & E). **A)** Mid-cartilaginous portion. **B)** Site of minimum cross-sectional area. **C)** Site of minimum circumference. **D)** Junctional portion. Shape of ET lumen was rather slitlike in both mid-cartilaginous portion (A) and site of minimum cross-sectional area (B), whereas it was rather wide and oval in two remaining sites. Although attachment (*) of tensor veli palatini muscle (TVPM) to lateral lamina (LL) of ET cartilage and Ostmann's fatty tissue (OF) were observed in both mid-cartilaginous portion (A) and site of minimum cross-sectional area (B), they were not seen in two remaining sites. L — lumen of ET, LVPM — levator veli palatini muscle, ML — medial lamina of ET cartilage.

the ET is probably unaffected by contraction of the TVPM,[5] the site of minimum circumference of the ET lumen is likely to be a critical determinant of ET stenosis in the open state. Hence, the anatomic sites of constriction of the ET lumen can be divided into 2 regions: a "kinetic" region that is opened by the TVPM and a "fixed" region that is unaffected by the TVPM. The kinetic region includes most of the cartilaginous portion of the ET; the fixed region includes the junctional and bony portions.

This division of the ET lumen into "kinetic" and "fixed" regions provides an insight into the potential predisposing factors for otitis media with effusion. Data presented by Suzuki et al[12] and this study demonstrate that the dimensions of the fixed portion of the ET do not differ significantly between children and adults. Our results also reveal that the extent of the narrowest portion of the ET lumen does not differ significantly between children and adults. However, the dimensions of the kinetic portion (the

DISTANCE, AREA, AND CIRCUMFERENCE IN SITES OF MINIMUM CROSS-SECTIONAL AREA AND MINIMUM CIRCUMFERENCE OF EUSTACHIAN TUBE

				Site of Minimum Cross-Sectional Area			*Site of Minimum Circumference*		
Case	*Age (y)*	*Sex*	*Group*	*Distance* (mm)*	*Area (mm^2)*	*Circumference (mm)*	*Distance* (mm)*	*Area (mm^2)*	*Circumference (mm)*
1	0.25	F	Child	14.92	0.30	4.66	15.56	0.51	4.51
2	0.67	F	Child	16.07	0.64	6.36	18.30	0.96	5.74
3	1.33	M	Child	16.69	0.61	5.19	17.63	0.74	5.04
4	4	M	Child	16.95	0.60	5.82	20.61	0.78	4.24
5	9	F	Child	14.89	0.90	7.84	17.33	1.13	7.23
6	10	M	Child	18.47	0.32	4.90	19.43	0.51	4.51
7	17	F	Adult	20.97	0.68	5.37	20.97	0.68	5.37
8	28	F	Adult	20.64	0.70	5.45	23.47	0.87	3.94
9	38	F	Adult	17.57	1.00	9.69	24.34	2.66	6.81
10	70	M	Adult	21.81	0.76	4.81	23.03	0.93	4.69
11	70	M	Adult	29.67	0.58	6.48	31.55	1.03	5.45
12	71	F	Adult	20.61	0.47	5.91	21.31	0.54	5.64
13	88	F	Adult	18.80	0.49	6.60	20.72	1.13	5.31
Average ± SD				19.08 ± 3.93	0.62 ± 0.20	6.08 ± 1.40	21.09 ± 4.05	0.96 ± 0.56	5.27 ± 0.96

*Distance from pharyngeal orifice of eustachian tube.

cartilaginous portion of the ET anterior to its narrowest part) differ markedly in both length and cross-sectional area. Hence, the fixed region can be regarded as the site of constant ET function both during development and during normal ET activity. The kinetic region, by contrast, can be regarded as the limiting factor for active clearance and ventilation of the middle ear and mastoid cavity.

When inflammation occurs in the ET and the nasopharynx, the ET lumen tends to be more collapsible than it is in the absence of inflammation.[13] Even if the narrowest portion of the ET lumen in the closed state has a relatively large circumference, it may collapse, and mucosal factors such as edema and a decrease in the normal mucous blanket containing surfactant may prevent the ET lumen from reopening. Therefore, an inflamed ET lumen with a large circumference may impede ET opening.

It is important to exercise caution in drawing physiological inferences from a temporal bone study using histologically sectioned specimens. Although we did not directly observe the shape of the ET lumen in the closed and open states, we estimated the location of the narrowest portion of the ET lumen on the basis of assumptions derived from endoscopic observations in the literature. The site of minimum cross-sectional area was used to estimate the location of the narrowest site along the crescent-shaped lumen in the resting state. Conversely, the site of minimum circumference was used to estimate the location of the site with the least potential cross-sectional area along the rounded lumen in the open state. Unexpected findings were that both sites were located in the cartilaginous portion of the ET and that the site of minimum circumference was shifted in the direction of the tympanic orifice relative to the site of minimum cross-sectional area. However, future studies with imaging methods will be needed to confirm the relative location of these sites during physiological conditions.

Artifacts such as tissue shrinkage cannot be avoided in a study using histologic processing of specimens.[14] This shrinkage has been estimated at 10% for tissues processed by the methods used in this study.[14] Thus, the measurements of the circumference and cross-sectional area of the ET lumen may have been underestimated.

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AUDITORY CONSEQUENCES OF RECURRENT ACUTE PURULENT OTITIS MEDIA

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To investigate whether recurrent purulent otitis media results in permanent hearing loss, we studied 2 subgroups of children from a cohort, earlier prospectively followed from birth to the age of 3 years. One subgroup had recurrent acute otitis media (n = 12), and the other had no acute otitis media at all ("healthy" children; n = 21). At follow-up of these subgroups at the age of 10, no child had acute otitis media or secretory otitis media. There was no difference between the groups in hearing level thresholds at the frequencies 125 Hz to 8 kHz. However, in the children with recurrent acute otitis media, as compared with the controls, the hearing levels at high frequencies (8 to 16 kHz) and the acoustic middle ear reflex thresholds were elevated, the middle ear compliance was higher, and click-evoked otoacoustic emission response levels and middle ear pressures were lower. The results suggest that the middle ear mechanics of children with recurrent acute otitis media are affected, and also that their cochlear function might be disturbed.

KEY WORDS — auditory consequences, follow-up, recurrent acute otitis media.

INTRODUCTION

More than 50% of Swedish children are reported to suffer at least 1 episode of acute otitis media (AOM) before the age of 4, and about 5% suffer frequent recurrences, with the highest incidence occurring between 6 and 24 months.[1] Between 40% and 70% of all children experience chronic or recurrent episodes of secretory otitis media (SOM).[2] The question of whether middle ear infection, which most frequently occurs in small children, can result in permanent hearing loss after the disease period has been a matter of controversy for a long time.[3-9]

Children who had repeated bilateral SOM during the first year of life, as diagnosed by pneumatic otoscopy and multiple auditory brain stem response (ABR) assessments, and are examined at 4, 6, and 9 years of age demonstrate deficits in the long term in some aspects of higher-order auditory processing (speech recognition in competition, visual-auditory learning, sound blending, auditory memory, masking level difference).[10]

Studies indicate elevated hearing level thresholds between 10 and 20 kHz in children with a history of recurrent acute otitis media (rAOM) or SOM as compared with healthy controls. This hearing loss has been proposed to have both conductive and sensorineural components.[6,8] In rAOM, the severity of the hearing loss seems to be related to the number of AOM episodes.[8]

The influence of early middle ear disease on early language development has been a matter of controversy. In a prospective study of ours, children with severe rAOM before the age of 3 exhibited no detectable delay in their language development when analyzed at 4 and 7 years of age, as compared with non-rAOM controls.[11] However, more recent investigations of speech perception and verbal memory in children with and without first-year histories of otitis media, tested at an age of 9 years, provided evidence of subtle long-term effects of early episodes of otitis media on auditory processing, phonological representations, and working memory.[12]

Among the common late sequelae of rAOM and SOM are atrophy, retraction, and scarring of the tympanic membrane — changes that are aggravated by protracted treatment with transmyringeal ventilation tubes.[13,14] Such changes could be expected to negatively influence middle ear mechanics and result in permanent hearing loss. However, permanent hearing loss at the conventionally studied frequencies is, as noted above, seldom seen after rAOM.[5,9] The influence of disease on middle ear function is usually studied by impedance audiometry, including tympanometry and middle ear reflexes.

Otoacoustic emissions (OAEs) are commonly defined as acoustic energy produced by the cochlea and recorded in the outer ear canal.[15,16] This definition implies a cochlear origin of the OAE generator. It is now commonly accepted that OAEs reflect an active process involved in the transduction stage of the auditory stimulus, related to the contractile activity ob-

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TABLE 1. CHARACTERISTICS OF SUBGROUPS

	Recurrent Acute Otitis Media Group (n = 13)	*Control Group (n = 29)*
Episodes of acute otitis media before age of 3 y	≥6*	0
Other upper respiratory tract infections before age of 3 y	Not specified	<6

*Mean, 12.3; range, 6 to 21.

served for the outer hair cells.[16]

One of the subclasses of evoked OAEs is termed transiently evoked emissions or click-evoked OAEs (CEOAEs), because the responses are commonly elicited by the use of short, broadband click stimuli. The available equipment provides on-screen information regarding the levels, frequency spectrum, and reproducibility of the OAE response. The CEOAEs are used clinically for screening and monitoring of cochlear function. The stimulus applied travels from the ear canal to the cochlea, and the elicited response is conducted in the reverse direction, to be recorded in the ear canal. The whole process of OAE recording is therefore vulnerable to external factors influencing the properties of the entire sound pathway, as well as the properties of the perceptive and emitting structures of the cochlea.[16,17] Any external changes influencing the middle and inner ear can affect the OAE response.

Speech discrimination is an important element of the hearing process. In disrupted speech audiometry, the discrimination scores are calculated relative to the number of correctly repeated key words in each sentence presented. In healthy young adults, the normal score range is 90% to 100%, depending on the number of interruptions per second (chopping frequency). Poor discrimination scores are found in patients with reduced speech understanding and central hearing disorders.[18]

The present study was conducted on a small but well-defined and well-documented, prospectively followed group of children. The aim of the study was to assess the extended functional effects of rAOM on the middle and inner ear. The tools applied to detect such effects were hearing level thresholds in the range 125 to 16,000 Hz, changes in OAE response, middle ear admittance, and disrupted speech audiometry.

SUBJECTS AND METHODS

Study Design. A cohort of 113 children recruited from Lund, Sweden, was prospectively followed from birth to the age of 3, clinically and through blood samples. The children were followed continuously at the hospital with careful registration of all AOM and other respiratory tract infection episodes.[19] The AOM was diagnosed otomicroscopically and defined as an acute episode of earache in a child with red bulging eardrum(s), occasionally febrile and with signs of upper respiratory tract infection. (A relapse during antibiotic treatment or within 2 days of its completion was not counted as a new AOM episode.) We defined rAOM as 6 or more episodes of purulent AOM, unilateral or bilateral, during a 12-month period.

In this cohort, 2 subgroups were discerned. The first subgroup (n = 13) comprised children with rAOM ("rAOM children"), and the second subgroup (n = 29; "controls") comprised children with no AOM and fewer than 6 other upper respiratory tract infection episodes during the study period[19] (Table 1).

The parents of the rAOM children and controls were contacted and asked to allow their children to participate in a follow-up investigation when they had reached the age of about 10 years. Of the 13 children in the rAOM subgroup, 12 (92%) were available for the 10-year follow-up, as were 21 of the 29 controls. The parents were interviewed by one of the authors regarding their children's disease history after the age of 3, with the focus on middle ear disease and hearing problems.

The children's medical records were collected from the otolaryngological, pediatric, surgical, and orthopedic departments and primary care centers and were scrutinized to determine the number of AOM and SOM episodes, the number of ventilation tube (VT) insertions before and after the age of 3, other illness episodes, and hospital admissions. The study was approved by the Ethics Committee of the Medical Faculty of Lund University, Sweden. At follow-up examination of the children's ears and hearing, neither the examining physician nor the audiologist was informed as to which subgroup the children belonged to.

Audiometry Tests. The audiometric test equipment was calibrated according to ISO standards 389 and 7566,[20] reference zero, for the calibration of pure tone air conduction audiometers and pure tone bone conduction audiometers.

Extended High-Frequency Audiometry. The extended high-frequency (HF) hearing levels were tested in a soundproof booth with an Entomed SA 210 audiometer and Sennheiser HDA 200 earphones. Air conduction hearing levels were individually determined for both ears at the frequencies 8, 9, 10, 11, 12, 13, 14, 15, and 16 kHz, tested in that order.[21] The audiometer was calibrated in accordance with data given by the Physikalisch-Technische Bunde-

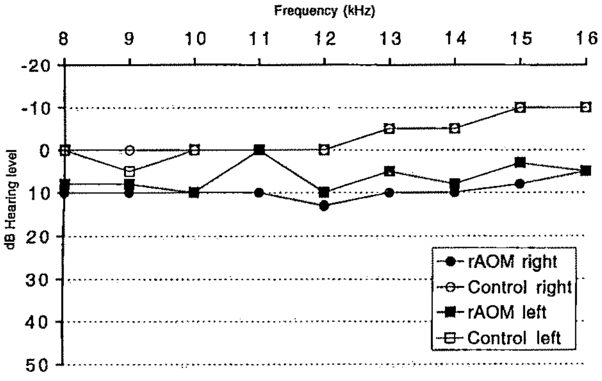

Fig 1. Median high-frequency hearing thresholds at 8 to 16 kHz in right and left ears in 12 children with recurrent acute otitis media (rAOM) and 21 controls. (See text for definitions.)

sanstalt.[22] The HF bone conduction levels were not measured.

Impedance Audiometry. A manually controlled Grason Stadler GSI 1723 middle ear analyzer was used for tympanometry and recording of the middle ear reflexes. For tympanometry, the probe frequency was 220 Hz, with a positive-to-negative sweep rate of 150 daPa/s from +2 kPa to −2 kPa. The immittance values obtained were recorded as tympanometric peak pressures in decapascals, and the admittance in milliliters equivalent volume was obtained by subtracting the baseline value from the compliance maximum. The compliance values were obtained in milliliters equivalent volume. The middle ear reflex thresholds were recorded during contralateral stimulation at 0.5, 1, and 2 kHz. The instrument was calibrated according to the recommendations in the manufacturer's instructions. The contralateral earphone was, however, calibrated according to ISO 389.[20]

Distorted Speech Audiometry. The intelligibility of distorted speech was tested bilaterally with the conventional audiometer and earphones described above. The speech was chopped at a frequency of 7/s and presented at 35 dB hearing level or at the most comfortable sound level. Normal test results for healthy young listeners for the type of test material used are considered to be in the range of 95% to 100%.[18]

Transient Click-Evoked Otoacoustic Emissions. The Otodynamics ILO 88 OAE analyzer with the corresponding software v.4.20B was used in default setup in the nonlinear mode. The responses were recorded with computerized averaging procedures time-locked to the transient stimulation. The reproducibility of 2 averaged waveforms was tested by a cross-correlation technique. The CEOAEs of both ears were recorded. The OAE responses were analyzed and compared globally, as well as separately, in the 5 frequency bands (1, 2, 3, 4, and 5 kHz) as presented by the ILO software.

Statistical Methods. Group comparisons of data for the different characteristics were performed with the Mann-Whitney U-test and Fisher's exact test.

RESULTS

The results of the clinical follow-up and of the pure tone audiometry between 125 and 8,000 Hz have been previously published.[9] The mean age of the 12 children in the rAOM subgroup was 10.8 years at the follow-up, and the mean age of the controls was 11.0 years. The total number of AOM episodes from birth to 10 years of age was 210 in the rAOM subgroup (mean, 17.5; range, 6 to 38). Between 3 and 10 years of age, the number was 62 in the rAOM subgroup (mean, 5.2; range, 0 to 17), as compared to 28 for the controls (mean, 1.3; range, 0 to 9; $p < .05$).[9] Protracted episodes of SOM were significantly more common in the rAOM subgroup than in the controls (6/12 versus 1/21; $p < .05$). One of the rAOM children still had a unilateral VT at the age of 10. At follow-up, otomicroscopic abnormalities (myringosclerosis, flaccid tympanic membranes) were seen in 8 of 12 (67%) rAOM children, as compared with 1 of 21 (5%) controls ($p < .001$).

TABLE 2. HIGH-FREQUENCY HEARING THRESHOLDS IN DECIBELS

	Right Ear					*Left Ear*				
	rAOM Group (n = 12)		*Controls (n = 21)*			*rAOM Group (n = 12)*		*Controls (n = 21)*		
Frequency (kHz)	*Median*	*Range*	*Median*	*Range*	*p*	*Median*	*Range*	*Median*	*Range*	*p*
8	10	−5 to +55	0	−5 to +25	NS	8	0 to +20	3	−5 to +15	<.05
9	10	−5 to +40	0	−5 to +20	<.05	8	−5 to +25	4	−5 to +15	<.05
10	10	−5 to +30	0	−5 to +30	<.05	10	−5 to +30	0	−5 to +25	<.001
11	10	−5 to +20	0	−5 to +10	<.01	0	−5 to +25	−1	−5 to +20	NS
12	10	+5 to +30	0	−5 to +30	<.001	10	−5 to +30	−1	−10 to +15	<.001
13	10	−5 to +35	−5	−15 to +25	<.001	5	−5 to +40	−4	−15 to +15	<.01
14	10	−20 to +50	−5	−15 to +20	<.01	8	−5 to +45	−4	−20 to +15	<.001
15	8	−20 to +40	−10	−20 to +15	<.01	3	−15 to +35	−5	−20 to +20	<.05
16	5	−25 to +50	−10	−25 to +10	NS	5	−25 to +35	−8	−25 to +10	<.05

rAOM — recurrent acute otitis media, NS — not significant.

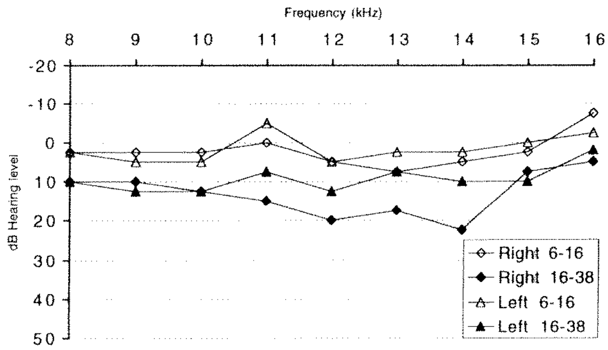

Fig 2. Median high-frequency hearing thresholds at 8 to 16 kHz in right and left ears in 6 rAOM children with 6 to 16 AOM episodes, and in 6 with 16 to 38 AOM episodes.

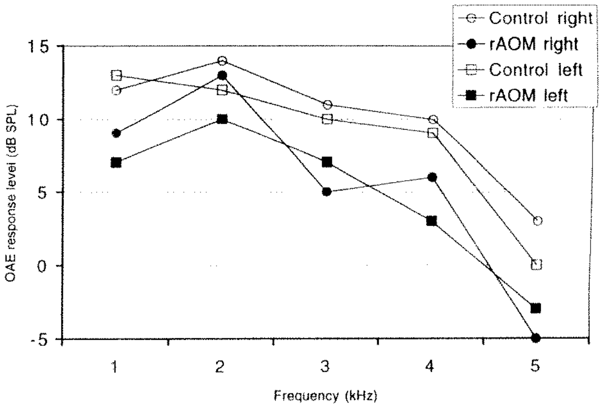

Fig 3. Median click-evoked otoacoustic emission (OAE) responses from right and left ears at 1 to 5 kHz in 12 rAOM children and 20 controls. (See text for definitions.) SPL — sound pressure level.

Audiological Tests. All data were analyzed separately for the right and left ears to avoid statistical dependence. In earlier published results, there was no significant difference between the subgroups regarding any of the hearing level thresholds for the frequencies 125 Hz to 8 kHz, and there were no air-bone gaps.[9]

Extended High-Frequency Audiometry. The rAOM children exhibited significantly higher hearing level thresholds at most frequencies measured as compared with the controls: in the right ears, at the frequencies 9, 10, 11, 12, 13, 14, and 15 kHz, and in the left ears, at the frequencies 8, 9, 10, 12, 13, 14, 15, and 16 kHz (Fig 1 and Table 2). The rAOM children were then divided into 2 groups (n = 6 in each) according to the number of AOM episodes from birth until follow-up: a group with 6 to 16 attacks and a group with 16 to 38 attacks. Two children had had exactly 16 attacks; one of them had experienced more bilateral AOM episodes and more episodes before the age of 3 than the other child and was assigned to the group with the most AOM episodes. Comparing these 2 groups of rAOM children, we find that those with the higher number of AOM episodes generally had higher hearing threshold levels than those with a lower number of AOM attacks; however, this was only statistically significant at 11 and 12 kHz on the right side and at 11 kHz on the left side ($p < .05$, respectively; Fig 2). The group with 6 to 16 episodes was also compared with the controls and exhibited significantly higher hearing level thresholds than the controls at 12 and 13 kHz in the right ears and at 10, 12, 13, 14, and 15 kHz in the left ears ($p < .05$).

Impedance Audiometry. At tympanometry, the maximum compliance on both sides was higher in the rAOM children. In the right ears, the median value was 2.7 mL in the rAOM children (range, 0.7 to 5.5 mL) and 1.3 mL in the controls (range, 0.6 to 4.5 mL; $p < .05$). In the left ears, the maximum compliance was 1.6 mL in the rAOM children (range, 0.8 to 5.8 mL) and 1.3 mL in the controls (range, 0.4 to 4.3 mL; $p < .05$). The middle ear pressure was significantly lower in the rAOM children than in the controls, as illustrated by the median values, which were −30 daPa (range, +20 to −250 daPa) and 0 daPa (range, +50 to −50 daPa) on the right side ($p < .01$) in the rAOM group and −20 daPa (range, +30 to −220 daPa) and +20 daPa (range, +40 to −35 daPa) on the left side ($p < .05$) in the controls. Two rAOM children exhibited bilateral middle ear pressures below −100 daPa (−250 daPa right, −220 daPa left, and −160 daPa on both sides), and 1 exhibited a unilateral pressure below this level (−130 daPa right).

When we measured the acoustic middle ear reflexes, we excluded the right ear of 1 of the rAOM children because of the presence of a VT. Thus, 11 right ears and 12 left ears were analyzed at the frequencies 0.5, 1, and 2 kHz. Reflexes could not be evoked at any frequency on either side in 3 of the 12 rAOM children at contralateral stimulation. In contrast, reflexes could be evoked in all 21 controls, except for 2 frequencies in 1 child ($p < .05$). Among the ears of the rAOM children in which acoustic reflexes could be evoked, the reflex threshold values were higher at 3 of 6 frequencies measured (at 2 kHz on the right side and at 0.5 and 1 kHz on the left side) as compared with the controls ($p < .05$ at each frequency).

Distorted Speech Audiometry. The mean discrimination of distorted speech was, on the right side, 67% in rAOM children and 72% in the controls, and on the left side, 72% and 73%, respectively (differences not significant).

Transient Click-Evoked Otoacoustic Emissions. For every child in both groups, a CEOAE response was recorded from either ear. In a few children, the responses were weak, with −5 dB being the lowest value recorded. If no recorded value was available, ie, the signal was below the noise level, when the OAE response was analyzed in the 5 frequency bands, the value was arbitrarily set at –6 dB for statistical purposes. The CEOAEs could be measured at most frequencies analyzed (1 to 5 kHz) in all of the 12 rAOM children and in 20 of the 21 controls. The ranges of the averaged responses for the right ear were –5 to 13 dB sound pressure level (SPL) in the rAOM group and 3 to 14 dB SPL in the control group. For the left ear, the corresponding values were –3 to 10 dB SPL in the rAOM group and 0 to 13 dB SPL in the controls. After separating the response into the 5 frequency bands, we found the right ear response to be significantly weaker in the rAOM group as compared to the controls in the frequency bands 4 and 5 kHz ($p < .05$). Similarly, the left ear response was weaker in the frequency bands 1, 4, and 5 kHz in the rAOM group ($p < .05$; Fig 3). For half of the children in the rAOM group and for only 1 of the controls, the frequency analysis failed to elicit a reponse at 5 kHz for the right ear ($p < .01$). For the left ear, a similar tendency was observed, but the difference between groups was not statistically significant.

Reduced middle ear pressure and weak OAE responses seemed to be correlated. In order to investigate the effect of negative middle ear pressure on the results, we excluded the 3 rAOM children with negative middle ear pressure exceeding –100 daPa in at least 1 ear from the statistical calculations. Consequently, no statistically significant difference between the OAE responses from the rAOM group and those from the controls could be confirmed for any of the 5 frequency bands.

In the rAOM children, no CEOAE response was seen at 5 frequencies in 1 child, at 4 frequencies in 2 children, at 2 frequencies in 1 child, and at 1 frequency in 1 child. In the controls, no response was seen at 3 frequencies in 1 child and at 1 frequency in another. One rAOM child who exhibited no CEOAE responses at any frequency on the right side had a bilateral middle ear pressure of –160 daPa. On the left side, the median response of this child was –5 dB. Another child with middle ear pressures of –250 daPa on the right and –220 daPa on the left showed no response at 3 frequencies. (On the right, the median response was –6 dB; on the left, it was –2 dB.) These were 2 of the 3 children with the highest negative middle ear pressures.

DISCUSSION

The principle upon which our present and earlier studies are based is to facilitate the identification of differences between small groups through adopting very strict inclusion criteria.[19] An advantage of relatively small groups is that the same physician can perform most of the otolaryngological examinations during the prospective part of the study, thus creating the greatest possible conformity in diagnosis and treatment. Every single AOM and SOM episode could thus be registered from birth until the age of 10.

Most studies on the effect of rAOM and SOM on hearing usually include 1 or more of the following tests: pure tone audiometry, tympanometry, or CEOAEs.[4,7,8,23] In the present investigation, HF audiometry, tympanometry, recording of CEOAEs, and speech tests were performed on the same children. As have other authors, we found significantly poorer HF hearing in the rAOM children than in controls, in spite of a normal conventional audiogram.[7,8] As have others, we found that a greater number of AOM episodes resulted in significantly higher median HF thresholds.[8] Higher compliance of the tympanic membrane, lower middle ear pressure, and higher otoacoustic reflex threshold levels in the rAOM group indicate an influence of disease on middle ear mechanics, including the functions of the eardrum, ossicles, and round and oval window membranes.

Whether the poor HF hearing and poor CEOAE responses in the rAOM children are the result of middle ear disease or have a cochlear origin is unknown. The reliability of HF testing has also been criticized.[7] Our observation of a relationship between the HF hearing threshold levels and the number of AOM episodes provides no conclusive evidence in favor of either cochlear or middle ear disease. It is well known that conductive hearing loss, ie, middle ear fluid (pus or mucoid secretion), impairs the CEOAE response.[23] Changes in middle ear pressure influence the recording of CEOAEs, and a middle ear pressure of –300 mm $H2O$ probably makes the CEOAEs unmeasurable.[16,24] Type B tympanograms combined with secretion in the middle ears do not, however, necessarily lead to the absence of recordable CEOAEs.[23] We found that the CEOAEs at several frequencies were significantly poorer in rAOM children than in controls in spite of normal hearing thresholds in the range 125 Hz to 8 kHz. However, when the rAOM children with the lowest middle ear pressures were excluded, there was very little difference in CEOAE levels between the rAOM group and the controls; this finding can be ascribed partly to the low pressure, but also partly to an effect of the small size of the groups.

It cannot be excluded that the vibratory qualities of the round and oval windows could be affected by repeated infections. The absence of both the effect of middle ear fluid and conductive hearing loss in the frequency range 125 Hz to 8 kHz suggests that some of the HF hearing loss of the rAOM children is of cochlear origin. Paparella et al[4] have demonstrated both temporary threshold shifts and permanent threshold shifts in patients and animals with purulent AOM. The pathophysiology of such a hearing loss in otitis media is proposed to be transmission of toxins and other bacterial products through the round window membrane, damaging the hair cells of the basal turn of the cochlea.[4,7]

No difference between rAOM children and controls was seen regarding the discrimination of distorted speech. Both groups, however, exhibited poorer discrimination than normal young adults,[18] probably because of the lower age of the children than those in the reference group.

The HF hearing loss has, by some authors, been said to be of no practical importance as long as the hearing is normal at the conventional frequencies.[5] However, as suggested by Hunter et al,[7] such a hearing loss may progress with age, ultimately influencing the conventional frequency region and possibly affecting speech perception in high levels of background noise. Higher-order auditory processing seems also to be affected in children with early rAOM and may have importance for classroom performance in the longer term.[10] The results of this study demonstrate the importance of careful clinical follow-up of rAOM children until the frequent AOM and SOM episodes have come to an end. It will be interesting to determine the functional relevance of the results in later follow-up studies of these subjects as adults.

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LONGITUDINAL AND CROSS-SECTIONAL PHENOTYPE ANALYSIS IN A NEW, LARGE DUTCH DFNA2/*KCNQ4* FAMILY

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We analyzed hearing thresholds, speech recognition scores, and vestibular responses in 32 affected persons in a large family with DFNA2/*KCNQ4*-related hearing impairment caused by a W276S missense mutation. Linear regression analysis of individual longitudinal data revealed significant threshold progression (1 dB/y) and offset (at age zero). The mean offset thresholds were 5, 21, 40, 39, 31, and 51 dB hearing level (HL) at 0.25, 0.5, 1, 2, 4, and 8 kHz, respectively. Cross-sectional analysis of last-visit thresholds against age produced less-steep slopes and higher offset thresholds. Nonlinear regression analysis of last-visit phoneme recognition scores against age in 25 cases showed that speech recognition did not deteriorate before the third decade. A hyperactive vestibulo-ocular reflex was found in 3 of 11 cases; 2 persons were especially susceptible to motion sickness. Persons with this *KCNQ4* mutation showed congenital, progressive high-frequency impairment without substantial loss of speech recognition during the first decades of life.

KEY WORDS — autosomal dominance, DFNA2, *KCNQ4*, sensorineural hearing impairment, speech recognition, vestibule.

INTRODUCTION

In 1994, a second locus for nonsyndromic autosomal dominant sensorineural hearing impairment, DFNA2, was identified in an Indonesian family and in an American family.[1] Since then, 10 families have been studied with progressive high-frequency sensorineural hearing impairment linked to the DFNA2 region on chromosome 1p34.[2-6] This finding probably reflects the important role of this locus in autosomal dominant inherited hearing impairment.[2] Furthermore, several different genes appear to occupy the DFNA2 region. The connexin 31 gene (*GJB3*) was the first to be identified.[3] The second was *KCNQ4*, which encodes a voltage-gated potassium ion (K^+) channel that is preferentially expressed in the outer hair cells of the cochlea.[4] Mutations in *KCNQ4* have been found in 7 unrelated families originating from Belgium, France, the Netherlands, and the United States. Most mutations caused changes in the P-loop domain (pore region) of the expressed protein. In contrast with the Indonesian family linked to DFNA2,[7] all of the families that were sufficiently studied had fairly similar phenotypes.[8,9] Both *GJB3* and *KCNQ4* may play a role in the K^+ recycling pathway of the inner ear.[9] It is probable that a third, as yet unidentified, DFNA2 gene exists, because the affected members of the Indonesian family did not show *GJB3* or *KCNQ4* mutations.[7] The involvement of 3 different genes is intriguing, but not exceptional, given the extreme heterogeneity of nonsyndromic hereditary hearing impairment. An up-to-date review of all genetic information can be found on the Hereditary Hearing Loss Homepage.[10] The progressive type of high-frequency impairment found in DFNA2 resembles presbycusis. It has therefore been suggested that studying this type of hearing impairment may provide more insight into the pathophysiology of presbycusis.[2]

Preliminary phenotypic analysis of the present family suggested linkage to the DFNA2 region, and subsequent genetic analysis revealed a missense mutation in the *KCNQ4* gene.[11] In this study, we present a detailed audiometric and vestibular analysis of this fourth Dutch DFNA2 family.

PATIENTS AND METHODS

A 5-generation pedigree was constructed with the aid of a family member (Fig 1). Eighty-six members of the family participated in this study after giving informed consent. They — or their parents, if they were too young — were asked to fill out a questionnaire to exclude other causes of hearing impairment. Special attention was paid to features of syndromic hearing impairment. All participants underwent otoscopic and audiometric examination and donated a

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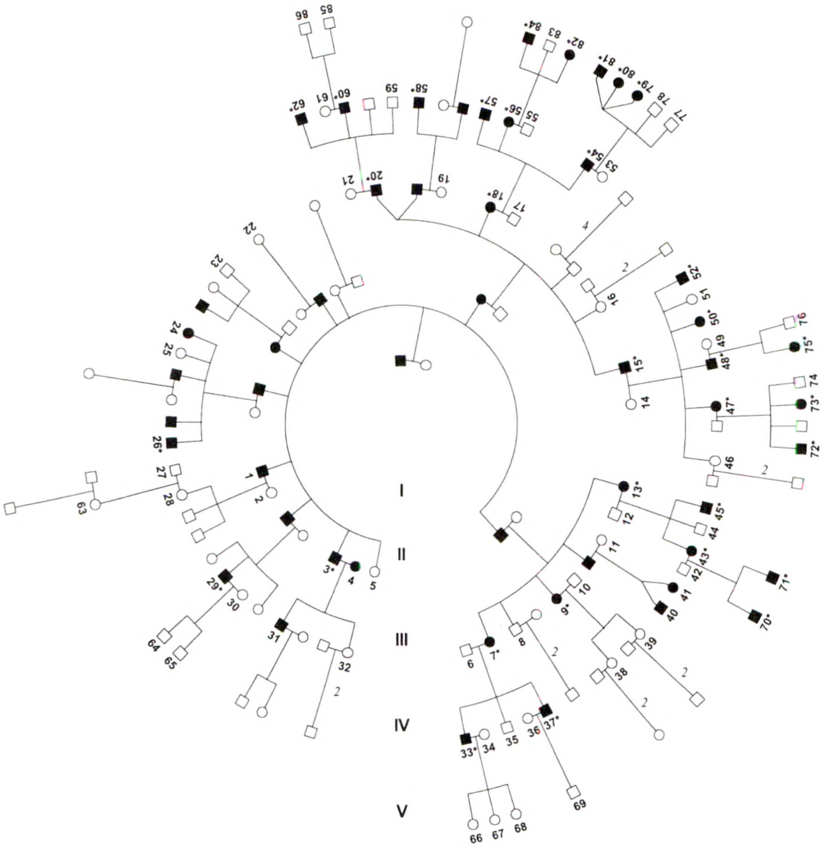

Fig 1. Family pedigree. Circles — women, squares — men. Filled symbols denote hearing impairment. Numbered persons participated in study. Mutation carriers are labeled with asterisks. Italics near descendant lines specify numbers of individuals.

blood sample. Pure tone and speech audiometry were performed in a sound-treated room according to current clinical standards. Tympanograms were only evaluated in incidental cases (glue ears). Computed tomographic scanning of the temporal bones and brain stem auditory evoked potential testing was performed in 1 affected person.

The present analyses were only performed on the patients for whom mutation analysis of the *KCNQ4* gene disclosed a W276S mutation.[11] We used the average audiogram slope over 5 octaves (from 0.25 to 8 kHz) to classify the audiogram configuration according to the following specifications: flat (slope < 5 dB per octave), gently sloping (5 dB per octave ≤ slope < 10 dB per octave), and steeply sloping (slope ≥ 10 dB per octave).

Longitudinal analyses included previously obtained audiograms. Using a commercial program (Prism, version 3.0; GraphPad, San Diego, California), we performed a linear regression analysis to evaluate progression in hearing impairment (pure tone thresholds) and to extrapolate the offset threshold (ie, the threshold at age zero). Out-of-scale measurements were excluded from the analyses. It was tested whether the correlation coefficient was significant, which implied that the regression coefficient, ie, slope — here called the annual threshold deterioration (ATD, in decibels per year) — differed significantly from zero. The Prism program's F test option (analysis of covariance) was used to determine which regression lines could be pooled. The program first tested between slopes, and it only tested between intercepts if the slopes were not significantly different at the $p = .05$ level. In each affected person, progression was considered to be significant if a significantly high number of frequencies out of the frequencies measured showed significant progression. Relative frequencies were labeled as significant if they were related to a tail probability of $p < .05$ in the appropriate binomial distribution ($n = 6$ for 6 frequencies, $p = .025$ for a significant positive correlation coefficient). The y-intercept — or offset (threshold) — was derived by extrapolation and subjected to statistical tests similar to those used for the slope. For lines with a negative y-intercept, it was tested whether the positive x-intercept (onset age) differed significantly from zero by calculating 95% confidence limits for the latter intercept by performing a nonlinear fit with the equivalent equation $y = \text{slope}\ (x - x_{\text{intercept}})$.

Cross-sectional analysis, comprising linear regression analysis, was also performed for the purpose of comparison, on only the last-visit threshold data.

Speech audiometry ($n = 25$) involved evaluating the speech reception threshold and the maximum recognition score (in a performance-intensity plot) for phonemes by use of standard, phonetically balanced

Dutch word lists. Cross-sectional analysis was performed on the most recent scores, with simultaneous measurement of sufficient pure tone thresholds (including 1, 2, and 4 kHz). The recognition scores for the right ear were fitted with a sigmoidal dose-response equation with variable slope against age, as well as the pure tone average (for the right ear) for 1, 2, and 4 kHz (PTA1,2,4 kHz), by use of a logarithmic scale for the x variable. These plots were compared to similar plots obtained previously at our clinic in a group of 45 patients with presbycusis alone (unpublished observations).

Vestibular examination was performed on 11 mutation carriers. Vestibular testing comprised rotatory and caloric stimulation with the patient in the dark (eyes open) and electronystagmography, including computer analysis, as previously described.[12-15] Response parameter values were labeled as significant at the 5% level. The group prevalence of significant findings was related to the corresponding binomial distribution (as described above) to see whether it was significant ($p = .05$).

RESULTS

We analyzed 33 clinically affected persons with a W276S mutation in the *KCNQ4* gene (labeled with an asterisk in Fig 1). One of them (patient 50) also had osteogenesis imperfecta and was therefore excluded from further analyses. Hearing impairment was also present in some non-carriers of the mutation (cases 1, 4, 24, 31, 40, and 41). Patient 1 had developed high-frequency hearing impairment already in the second decade. Patient 4 was related by marriage. Patient 24 had a history of meningitis. Patient 31 was the son of 2 hearing-impaired parents (patients 3 and 4). Patients 40 and 41 showed atypical audiograms. The non-numbered persons are those who did not participate in this study; some of them were judged to be affected on the basis of their history.

Figure 2 shows the pure tone audiograms of the mutation carriers. As all of the patients showed fairly symmetric audiograms, only the right ear was included (the left ear in case 81, because the right ear was not measured). The audiogram configuration was classified as gently sloping in 13 cases, steeply sloping in 16 cases, and flat in 3 cases. A flat configuration was found only in patients younger than 6 years (Fig 2; cases 71, 82, and 84). In case 48, the configuration was gently sloping from 5 to 12 years of age and steeply sloping at 38 to 40 years of age.

Figure 3 shows the longitudinal analyses (right ear threshold against age). Only patients with more than 3 measurements that covered a suitable age range were included. Significant progression (bold lines) was found at a significantly high number of frequencies in 6 of the 10 persons that could be evaluated. This indicates a significantly high prevalence (ie, $p < .05$ in a binomial distribution with $p = .025$ and $n = 10$), which provides evidence for systematic progression. In most cases, the slopes (ATD values) could be pooled for most of the frequencies (data not shown). These values were also used to obtain across-subject estimates of the ATD. The resulting values at 0.25 to 8 kHz were in the range of 0.8 to 1.4 dB/y and could be pooled (ATD = 0.99 dB/y) after excluding 6 (of the 50) lines with extreme slopes. For each frequency, the weighted, across-subject mean offset threshold (y-intercept) was calculated with a weight of 1/SE. All frequencies showed significant prevalence (from 2 of 4 cases to 6 of 10) of significant (>0) offset thresholds. Only 3 lines (of 50) related to a significant onset age; the values were 9, 19, and 24 years. A significant majority of the findings thus showed congenital hearing impairment at all frequencies.

Cross-sectional analysis of the last-visit thresholds of all cases produced a pooled ATD value of 0.6 dB/y (across-frequency range, 0.48 to 0.96 dB/y) and offset thresholds of 16, 30, 41, 48, 53, and 63 dB at the increasing frequencies (dashed lines in Fig 3; it should be realized that not all cross-sectional data are shown). The ATD values were thus substantially lower and the offset values were higher than the values pertaining to the (dotted) trend lines in the individual longitudinal analyses.

The speech reception threshold (data not shown) was generally in agreement with the pure tone audiogram. Cross-sectional analyses of the phoneme recognition scores are shown in Fig 4. Figure 4A shows the deterioration in speech recognition scores with increasing age in the DFNA2 patients (circles) and presbycusis patients (crosses). In the DFNA2 patients, speech recognition did not deteriorate substantially before the third or fourth decade (a 90% score was reached at 36 years of age; 95% confidence range, 26 to 49 years). Figure 4B shows that speech recognition in the DFNA2 patients deteriorated substantially only after a relatively high level of pure tone hearing impairment had developed. The 90% score was reached at about 66 dB HL (95% confidence range, 56 to 76 dB HL). The DFNA2 patients showed earlier and slower deterioration of speech recognition than did the presbycusis patients (Fig 4A). At similar PTA1,2,4 kHz levels, the DFNA2 patients tended to have somewhat higher scores than the presbycusis patients (Fig 4B). There was only a minor

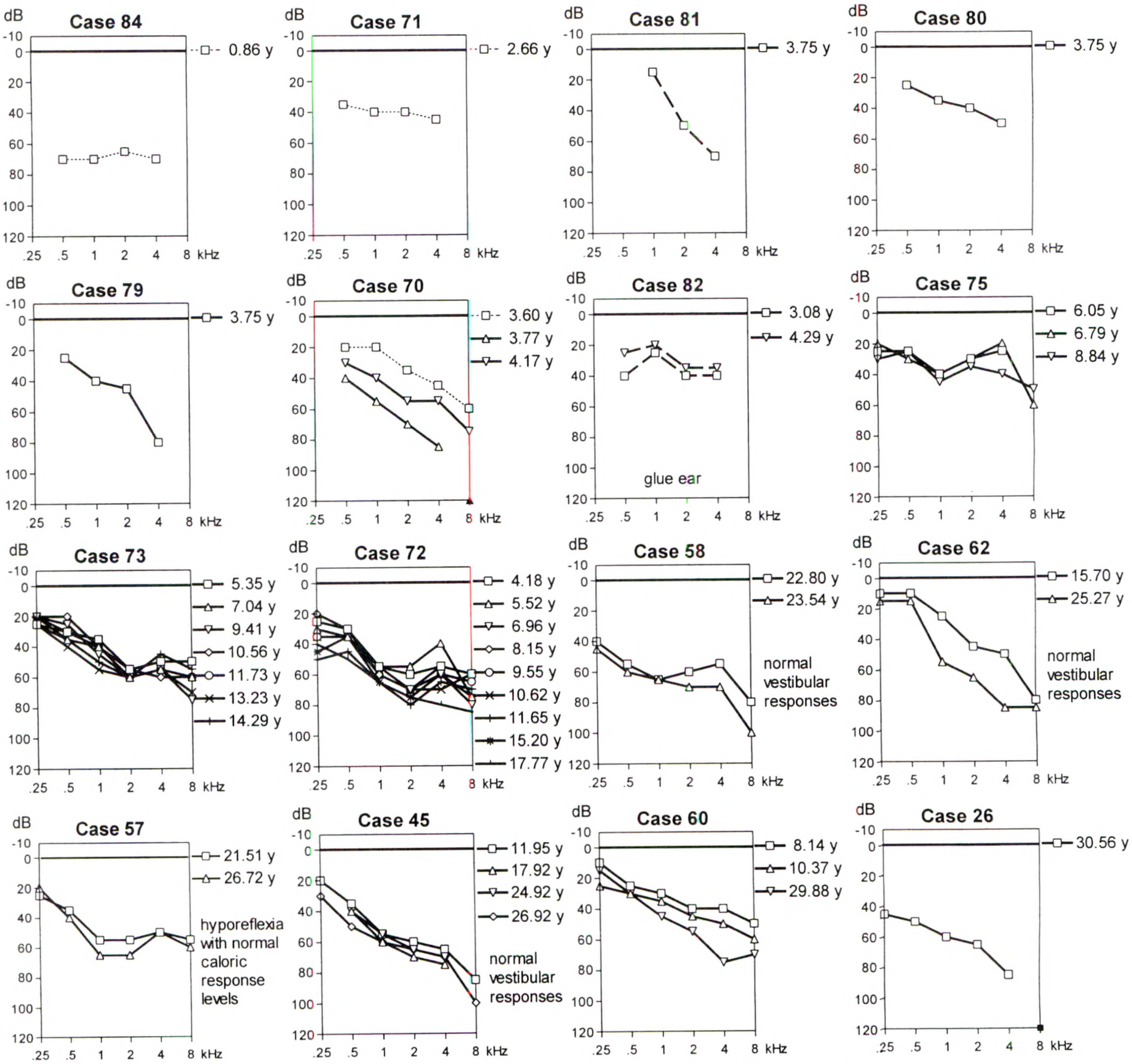

Fig 2. Pure tone hearing thresholds in decibels hearing level (HL) in right ear in 32 mutation carriers (case numbers in pedigree), including air conduction (continuous lines), free-field (dotted lines), and bone conduction (dashed lines) thresholds. Filled symbols at 120 dB HL indicate out-of-scale thresholds. Note that cases are ordered by age in years (y) at last visit. (Continued on next page.)

difference in slope between the two fitted curves.

Vestibular examination was performed in 11 affected persons. We found a significant prevalence of vestibular hyperreactivity (3 of 11 cases). Two of the persons with hyperactive responses were especially susceptible to motion sickness, but none of the other affected persons reported such susceptibility or balance problems. Two persons showed hypoactive rotatory responses (not significant), but had normal caloric responses.

Computed tomographic scanning showed a normal temporal bone configuration in case 43. The brain stem auditory evoked potentials in this patient were also normal.

DISCUSSION

The *KCNQ4* W276S mutation has hitherto been found to be the causative agent of hearing impairment in 3 families: 2 of Dutch origin and 1 of Japanese origin.[7,11] Haplotype analyses argued against a common ancestor of the Japanese family and the 2 Dutch families.[11] Position 276 in the *KCNQ4* gene is therefore a potential hot spot for mutation leading to progressive high-frequency hearing impairment.

In this study, we performed longitudinal analyses (Fig 3), as well as cross-sectional analysis (dashed regression lines in Fig 3), in this second Dutch fam-

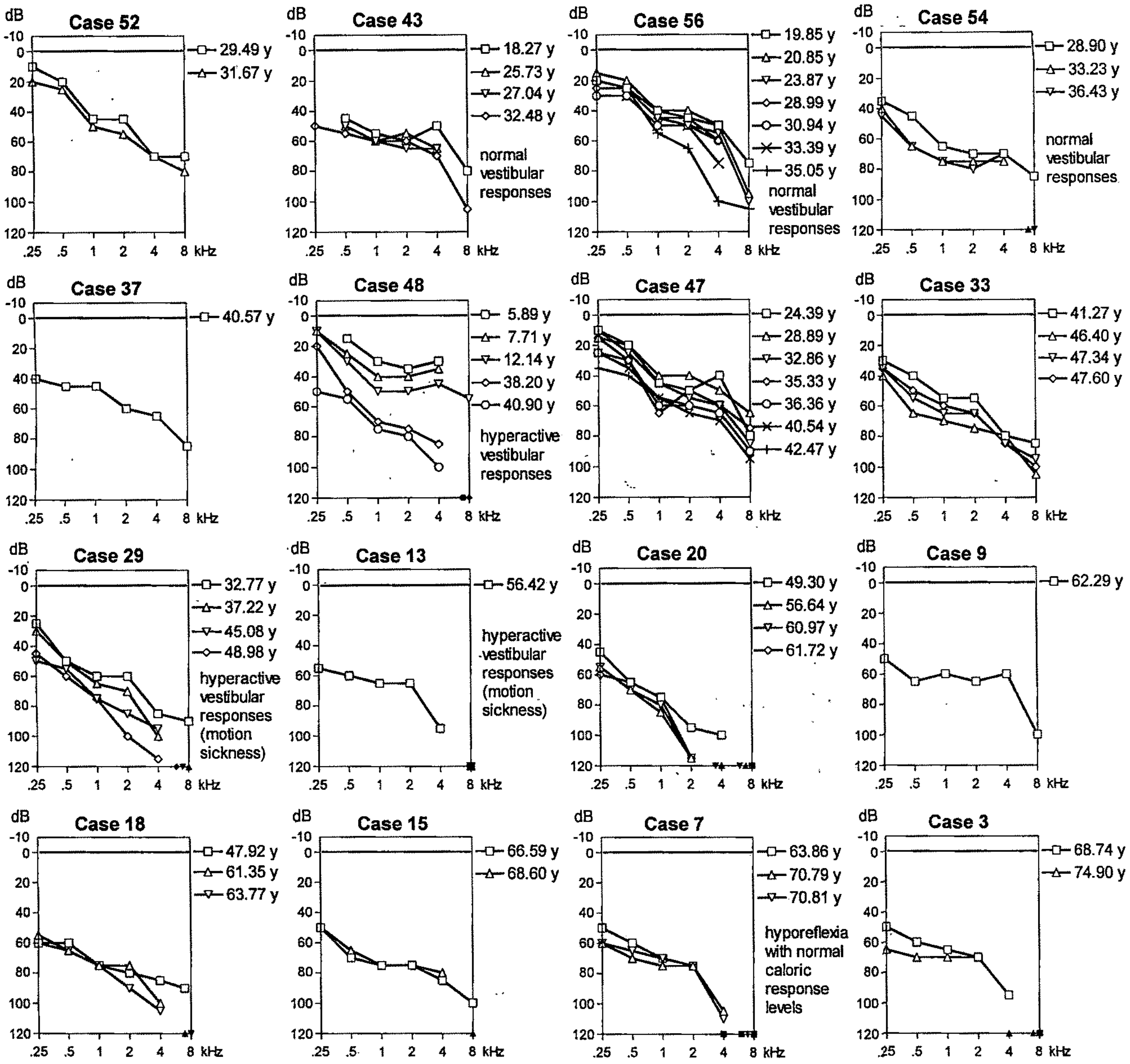

Fig 2, continued.

ily with the *KCNQ4* W276S mutation. Comparison of the results obtained with the two methods revealed a discrepancy that compromised the reliability of the cross-sectional analysis. A possible explanation can be sought in the large intersubject differences in offset level and/or onset age. Given the lack of sufficient longitudinal data in our previous DFNA2 studies, the possibility of similar bias in those cross-sectional data cannot be excluded. The sparse longitudinal data in previously reported DFNA2/*KCNQ4* families[16-18] did not differ substantially (data not shown) from the present data. As previously suggested, the higher ATD values in the longitudinal analyses may be related to the tendency for cases with more severe progression to undergo serial measurements. In the present family, suitable longitudinal data (n > 3) were available in 10 cases (Fig 3). We also inspected the data of all of the other cases (n = 22) and found no indications that these were substantially different from the available longitudinal data. Therefore, the suggested mechanism of (self-) selection does not seem to apply to the present family.

In this family, there was a tendency for excessively high thresholds to occur at a very young age (Fig 2). This finding can be questioned. Threshold measurement at a young age can be very difficult. The flat audiometric configuration seen only at a very young age may (also) have been caused by unreliable measurements.

As in our previous studies on Dutch families with

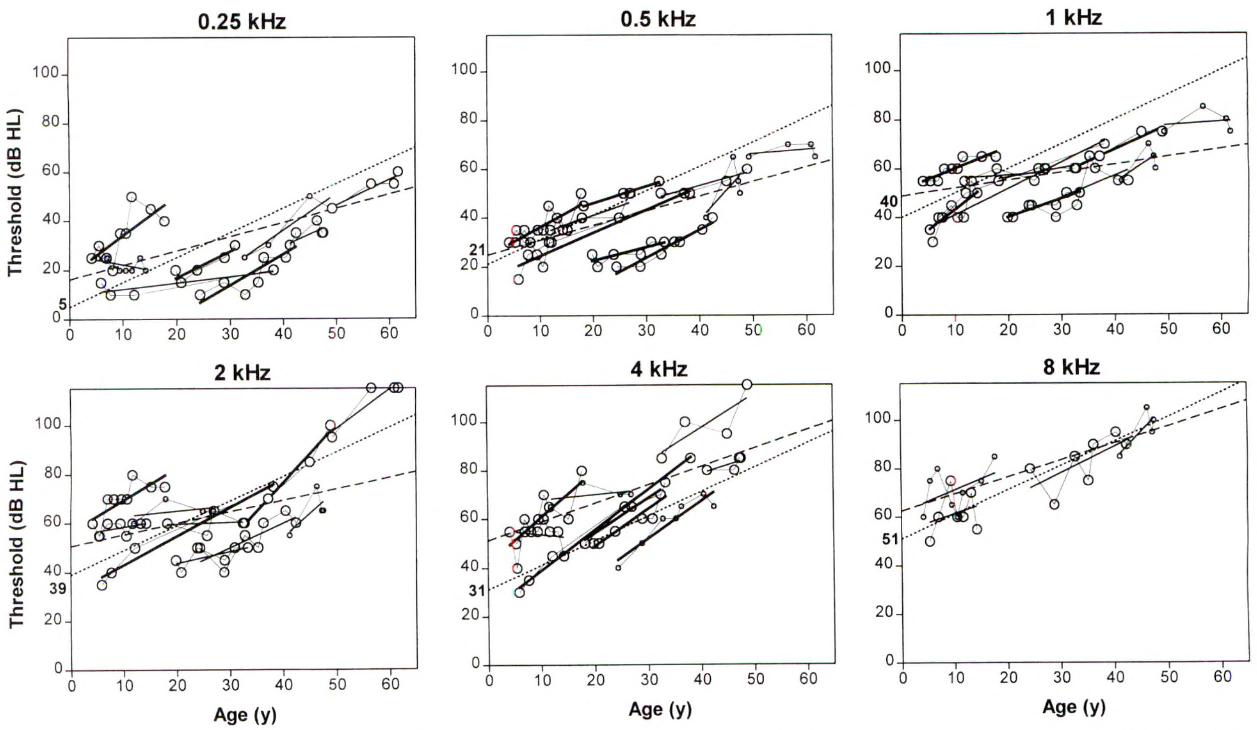

Fig 3. Longitudinal analyses at separate frequencies in 10 mutation carriers. Consecutive air conduction thresholds (right ear, dB HL) are plotted (circles connected with hair lines) against patient's age. Individual regression lines are included; bold line indicates significant progression. Dotted line is trend line with slope of 1 dB/y (ie, pooled slope of separate regression lines). Intercept of this line (bold figure near y-axis) was calculated as weighted mean of individual intercepts. Two individual regression lines with most extreme slopes (small circles) were excluded from this calculation. Dashed line in each panel is regression line obtained in separate cross-sectional analysis.

KCNQ4 mutations,[16-19] we did not find any indications of substantial nonlinearity of progression in hearing impairment, but the longitudinal nature of the present study lends more credibility to this contention. The present analyses clearly indicated steady, approximately parallel progression at all frequencies by an average of 1 dB/y (Fig 3).

In all of our previous reports on families with DFNA2-linked hearing impairment,[16-19] we mentioned the possibility of prelingual hearing impairment at the higher frequencies, but this could not be sufficiently substantiated with longitudinal data analyses. Some firm conclusions can be drawn from the present results. The statistical tests performed in individual cases clearly indicated that substantial hearing impairment (most prominent at the higher frequencies and amounting to 50 dB) was present already at a very young age and was most probably congenital. With an ATD of 1 dB/y, such individuals

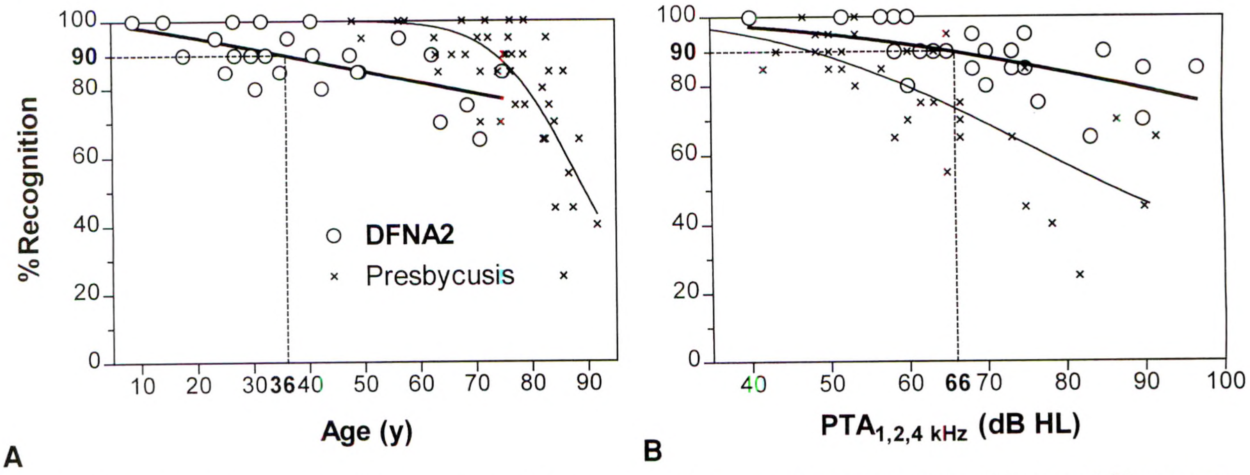

Fig 4. Cross-sectional analysis of binaural mean phoneme recognition scores in percent against **A)** age and **B)** pure tone average for 1, 2, and 4 kHz (PTA$_{1,2,4\ \text{kHz}}$) in 25 mutation carriers (circles and bold line) and 45 presbycusis patients (crosses and thin line). Sigmoidal curves were fitted with nonlinear equation.

will develop a total loss of about 50 + 60 = 110 dB by the age of 60 years. Experimental studies[4,20] have shown predominant cochlear expression of *KCNQ4* in the outer hair cells. Loss of outer hair cell function can cause a maximum of 60 dB hearing loss.[21] It can be suggested that congenital failure in outer hair cell function accounts for the observed (strictly speaking, derived) congenital threshold elevation. Further progression in hearing impairment may be explained in terms of secondary effects of the loss of outer hair cell function. Failure of K^+ efflux through the basolateral membrane might lead not only to poisoning of these cells,[20] but also to collateral damage. Failure of recycling of K^+ back to the endolymph might lead to failure of endolymph ion homeostasis and jeopardize the function of important structures such as inner hair cells and afferent cochlear neurons.

Marres et al[19] reported that a substantial proportion of patients (25% to 35%) with a W276S mutation in the *KCNQ4* gene showed vestibular hyperactivity in response to rotatory stimulation. Some of these patients were especially susceptible to motion sickness. None of the other patients had any relevant vestibular signs or symptoms. That was the only previous study on DFNA2 that included vestibular testing. Fairly similar observations were made in the present family. Kharkovets et al[20] recently found that *kcnq4* in mice is also expressed abundantly in type I vestibular hair cells, as well as in afferent vestibular neurons and their terminals in the vestibular nuclei. It therefore seems possible that our finding of vestibular hyperactivity in the 2 families with the W276S mutation can be explained on the basis of release from (central) inhibition.

This is the first report on DFNA2/*KCNQ4* that addresses both pure tone thresholds and speech recognition. Our cross-sectional analysis indicated that although hearing impairment was present prelingually, especially at the higher frequencies, postlingual progression had the most substantial influence on speech recognition (Fig 4A). Not unexpectedly, DFNA2 patients showed an earlier decrease in speech recognition scores than did presbycusis patients (Fig 4A). It should be noted that our presbycusis data may have been biased by (unavoidable) selection of patients with speech recognition problems, so the higher rate of deterioration in the presbycusis patients may reflect a bias of case selection. This possibility also applies to the observation that the DFNA2 group had better recognition scores at similar threshold levels (Fig 4B). The relatively good recognition scores in our DFNA2 patients would seem to indicate that there was no substantial involvement of central auditory pathways. Such involvement has been suggested in mice on the basis of expression studies.[20] Furthermore, we could not detect any abnormalities in brain stem auditory evoked potentials in the affected family member tested. Additional auditory tests on retrocochlear function should be performed in suitable cases to help clarify this issue in regard to DFNA2/*KCNQ4* patients.

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EXPRESSION OF HEAT SHOCK PROTEIN 70 IN THE COCHLEA IN EXPERIMENTAL AUTOIMMUNE INNER EAR DISEASE

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To explore whether the immune response of the inner ear could induce heat shock protein 70 (hsp70) in the guinea pig cochlea, we established a model of autoimmune inner ear disease (AIED) by systemically immunizing guinea pigs with homologous crude inner ear antigen (CIEAg).The immunized cochleas and normal control cochleas were examined for the expression of hsp70 by immunohistochemical analysis and in situ hybridization. It was found that 10 of 28 ears showed significant increases in hearing thresholds after the animals had been sensitized with CIEAg, and 67.9% of the ears from the immunized animals had modiolar vasculitis. The hsp70-like proteins were basically expressed in the normal cochleas, but dramatically increased expressions of hsp70 (perhaps also including hsp70-like molecules) and its messenger RNA were observed in the cochleas of the AIED models. The results suggest that the immune response of the inner ear induced the expression of hsp70 in the guinea pig cochleas.

KEY WORDS — autoimmune disease, cochlea, heat shock protein, sensorineural hearing loss.

INTRODUCTION

A specific inner ear antigen is needed to elucidate the pathogenesis of autoimmune inner ear disease (AIED). In 1990, Harris and Sharp[1] first detected an obviously elevated serum anti–68 kd protein in Western blots against bovine inner ear extracts and found that 35% of patients with rapidly progressive sensorineural hearing loss showed antibody reactivity. Other studies indicated that the 68 kd protein may represent heat shock protein 70 (hsp70).[2]

In this experiment, a homologous crude inner ear antigen (CIEAg) from guinea pigs and a well-defined immunization schedule were adopted to develop a guinea pig model of AIED. Immunohistochemical analysis and in situ hybridization were also used to detect the expression of hsp70 in the cochleas of control animals and the experimental animals with AIED. The purpose of this study was to determine whether the immune response of the inner ear could induce the synthesis of hsp70 in guinea pig cochleas.

MATERIALS AND METHODS

Animals. Twenty-four healthy young adult Hartley guinea pigs weighing 250 to 350 g, with a normal Preyer's reflex, supplied by the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology, were randomly divided into an experimental group (14 animals) and a control group (10 animals). For all experimental procedures, the animals were anesthetized with sodium pentobarbital (30 mg/kg).

CIEAg Preparation. A CIEAg was prepared from 20 guinea pigs. The animals were deeply anesthetized and then rapidly decapitated to obtain the tympanic bullae. After removal of the bony wall, the membranous labyrinth and the modiolar portion of the acoustic nerve were harvested. The inner ear tissue was homogenized with an ultrasonic cell destroyer in sterile chilled phosphate-buffered saline solution (PBS), and then the solution was centrifuged at 3,000 rpm for 10 minutes. The supernatant was collected and then lyophilized to obtain a dry CIEAg for the immunization procedure.

Immunization. Fourteen experimental guinea pigs were systemically immunized with CIEAg (200 µg/0.2 mL) in complete Freund's adjuvant. The animals were then given 7 booster doses of CIEAg (100 µg/0.2 mL) in incomplete Freund's adjuvant at 1, 2, 3, 4, 5, 6, and 7 weeks. The controls received the same schedule of immunization with only Freund's adjuvant. Ten days after the last boost, all animals were sacrificed to obtain their temporal bones for routine histologic and immunohistochemical analysis and in situ hybridization.

Electrophysiological Testing. The hearing of each animal was assessed before immunization to get a baseline recording and 7 days after the last boost. The auditory evoked brain stem response thresholds were measured bilaterally in all animals with surface electrodes according to the method described by Gong and Wang.[3]

Cochlear Processing. After anesthetization, all of

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TABLE 1. HEARING THRESHOLDS

		Hearing Thresholds	
Animals	*No. of Ears*	*Before Immunization*	*After Immunization*
Experimental	28	44.6 ± 4.3	54.1 ± 9.3*
Control	20	44.5 ± 3.9	44.5 ± 4.3

Data are mean ± SD in decibels peak sound pressure level.
*Comparison to before immunization: $p < .01$ (t-test).

TABLE 2. SHIFT OF HEARING THRESHOLD FOLLOWING SYSTEMIC CHALLENGE WITH CRUDE INNER EAR ANTIGEN

		Threshold Shift			
Animals	*No. of Ears*	*None*	*<10 dB*	*≥10 dB*	*Score**
Experimental	28	0	18	10	35.7%
Control	20	16	4	0	0%

*Percentage of ears with obvious hearing loss.

the experimental guinea pigs and controls were rapidly decapitated. The temporal bone was quickly removed, and the bone surrounding the cochlea was trimmed. To aid in penetration of the fixative — 4% paraformaldehyde in 0.01 mol/L PBS — the footplate of the stapes was removed from the oval window, the round window membrane was punctured, and a small hole was made in the cupula of the apex. The cochlea was then fixed for 4 hours, decalcified with 10% ethylenediaminetetraacetic acid, and cryoprotected, all at 4°C. After the cochlea sank in 30% sucrose, it was embedded in OCT compound (Miles Inc, Elkhart, Indiana). Cryosections (10 µm) were cut along the midmodiolar plane of each cochlea with a cryostat (Reichert Jung 1800, Nussloch, Germany) and collected on glass slides coated with 3-aminopropyltriethoxysilane (Boster Scientific, Wuhan, China). The sections were stored at −70°C.

Immunohistochemical Staining of Anti-hsp70 Antibody. Cryosections of the cochleas from the experimental animals with an elevation of hearing threshold of 10 dB or more (10 ears) and from the controls (10 ears, selected at random) were subjected to immunohistochemical staining. Ten ears with a hearing threshold shift of less than 10 dB from the experimental animals were also involved in this study.

The sections were treated with 3% hydrogen peroxide and blocked with normal goat serum. They were then incubated in mouse monoclonal anti-hsp70 (1:100; Sigma Chemical, St Louis, Missouri) at 37°C for 1 hour. Normal mouse serum was substituted for the monoclonal antibody in the sections that served as immunohistochemical controls. The antibody was detected by the streptavidin-biotin-peroxidase method (Boster Scientific) with diaminobenzidine hydrochloride as the chromogen. The sections were then dehydrated and coverslipped.

In Situ Hybridization of hsp70 Probe. All of the above cochlear sections (30 ears) underwent in situ hybridization.[4] The specific hsp70 oligonucleotide probe was commercially prepared and labeled with digoxin at the 5' termination (Sangon Chemical, Shanghai, China), with the sequence being 3'-GG TAC CAC GAC TGG TTC TAC TTC CTC TAG C-5'. Briefly, the in situ hybridization procedure was as follows. Sections were acetylated in 0.25% acetic anhydride in 0.1 mol/L triethanolamine in 0.9% sodium chloride, pH 8.0, for 10 minutes at room temperature. They were dehydrated in successive graded concentrations of alcohol (up to 100%), washed and air dried, and incubated with prehybridization solution (6× SSC [saline sodium citrate buffer], 50% deionized formamide, 5× Denhardt's solution, 100 µg/mL salmon sperm DNA, and 10% dextran sulfate). After prehybridization, each section was incubated with 20 µL of solution containing the hsp70 probe (2 µg/mL in prehybridization solution) and covered with a Parafilm coverslip. Hybridization was carried out in a humid chamber at 37°C for 17 hours, and then the sections were rinsed sequentially in 2× SSC, 0.1× SSC, and buffer I (1 mol/L sodium chloride, 0.1 mol/L Tris–hydrochloric acid, pH 7.5) for several times, and then blocked with buffer II (3% bovine serum albumin in buffer I). The sections were incubated with mouse monoclonal anti–digoxin–alkaline phosphatase (1:200; Boehringer-Mannheim Chemical, Mannheim, Germany) at 37°C for 1 hour. The phosphatase activity was developed with nitroblue tetrazolium (75 mg/mL) and 5-bromo-4-chloro-3-indolyl phosphate (50 mg/mL; Boehringer-Mannheim Chemical). The sections were then dehydrated and coverslipped.

Two types of controls were used to evaluate the specificity of the hybridization signal. Sections were treated with RNase A (200 µg/mL; Sigma Chemical) before hybridization at 37°C for 30 minutes, and sections were submitted to hybridization without the addition of the probe. The other procedures were the same as those mentioned above.

RESULTS

Electrophysiological Testing. In the experimental group, an elevation of hearing threshold (threshold shift) of 10 dB or more was recorded in 10 of 28 ears (35.7%; Tables 1 and 2). The change was unilateral in 6 animals and bilateral in 2. The threshold shift was 10 dB in 3 ears, 15 dB in 4 ears, 20 dB in 1 ear, 25 dB in 1 ear, and 40 dB in 1 ear. As compared with that measured before immunization, the hearing threshold of the experimental animals was significantly increased 1 week after the last boost ($p < .01$,

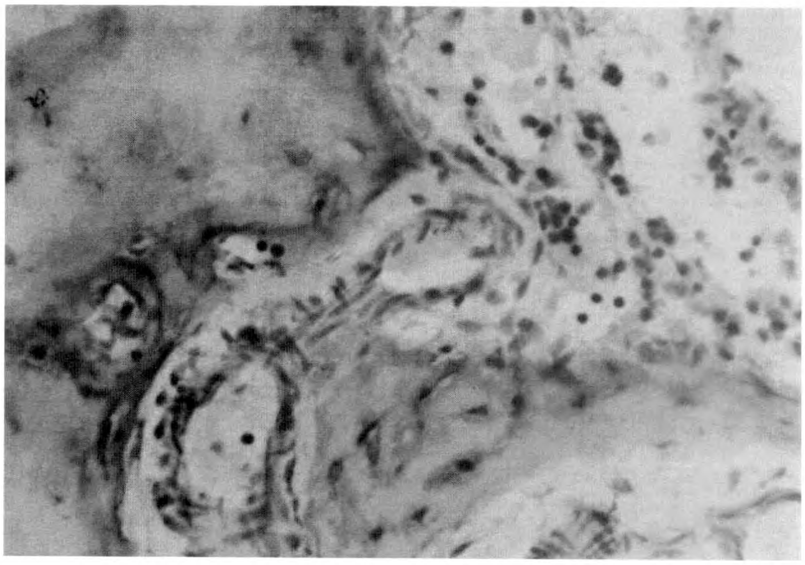

Fig 1. Moderate perivascular mononuclear cellular infiltration of modiolar vessels (H & E, original ×400).

t-test). The control guinea pigs, immunized with Freund's adjuvant only, had a change of 0 to 5 dB in hearing threshold (Tables 1 and 2).

Histopathologic Analysis. Modiolar vasculitis was observed in different grades in 67.9% of the ears from the experimental group (19 of 28 ears). The modiolar vessels appeared to have mild to moderate perivascular mononuclear cellular infiltration with lymphocytes and occasional plasma cells (Fig 1). Degeneration of the spiral ganglion cells could be found occasionally. Comparable pathological changes were not seen in the inner ears of the controls.

Immunohistochemical Staining of Anti-hsp70 Antibody. With the streptavidin-biotin-peroxidase technique, a faint hsp70-like immunostaining was seen in the cochlear cells (4 of 10 ears, 40%) of the control guinea pigs immunized with Freund's adjuvant only, with the staining mainly in the cytoplasm of interdental cells and spiral ganglion cells (Fig 2A). The cochleas from those experimental animals without an obvious hearing threshold shift, compared with the controls, showed an identical immunostaining pattern, and only a few immunoreactive stained interdental cells and spiral ganglion cells were seen. In the experimental animals with an elevation of hearing threshold of 10 dB or more, intense immunostaining was detected in 9 of 10 ears (90%), with the staining mainly present in the spiral ganglion cells (Fig 2B), organ of Corti (Fig 2C), stria vascularis, and spiral ligament. There was no positive staining in immunohistochemical control sections incubated with normal mouse serum only.

In Situ Hybridization of hsp70 Probe. In the cochleas of control and experimental animals without an obvious hearing threshold shift (<10 dB), very few hybridization signals were found over the tissues. In experimental guinea pigs with an obvious elevation of hearing threshold (≥10 dB), strong expression of hsp70 messenger RNA was present in all 10 ears (100%), and the positive staining indicative of hsp70 messenger RNA was present in the cytoplasm of spiral ganglion cells (Fig 3A), organ of Corti, stria vascularis, and spiral ligament (Fig 3B). No positive staining appeared in control sections incubated without hsp70 probe or pretreated with RNase.

DISCUSSION

Since McCabe's[5] original 1979 description of an autoimmune disorder that preferentially affects the ear, considerable evidence has been found to support this entity. Attempts have been made to identify specific inner ear antigens with which circulating antibodies and active lymphocytes react in patients with this disease. In 1990, Harris and Sharp[1] first identified the presence of a serum antibody against a 68 kd protein in Western blots against bovine inner ear extracts. They found that 35% of sera from patients with rapidly progressive sensorineural hearing loss showed antibody reactivity against the 68 kd inner ear protein. Some patients with antibody reactivity also showed positive responses to steroid treatment. Zou et al[6] also found a 68 kd protein in the guinea pig inner ear. In other studies, several antibodies reactive with inner ear–specific antigens or non–organ-specific antigens were found,[7-9] but the 68 kd protein was the most common antigenic target in patients with rapidly progressive sensorineural hearing loss.[10] The antibody against the 68 kd bovine inner ear antigen was also found in the sera of 30% of a group of patients with Meniere's disease.[11] In an investigation by Billings et al,[2] it was shown that the 68 kd bovine inner ear protein may represent hsp70.

Heat shock proteins fulfill cellular functions that

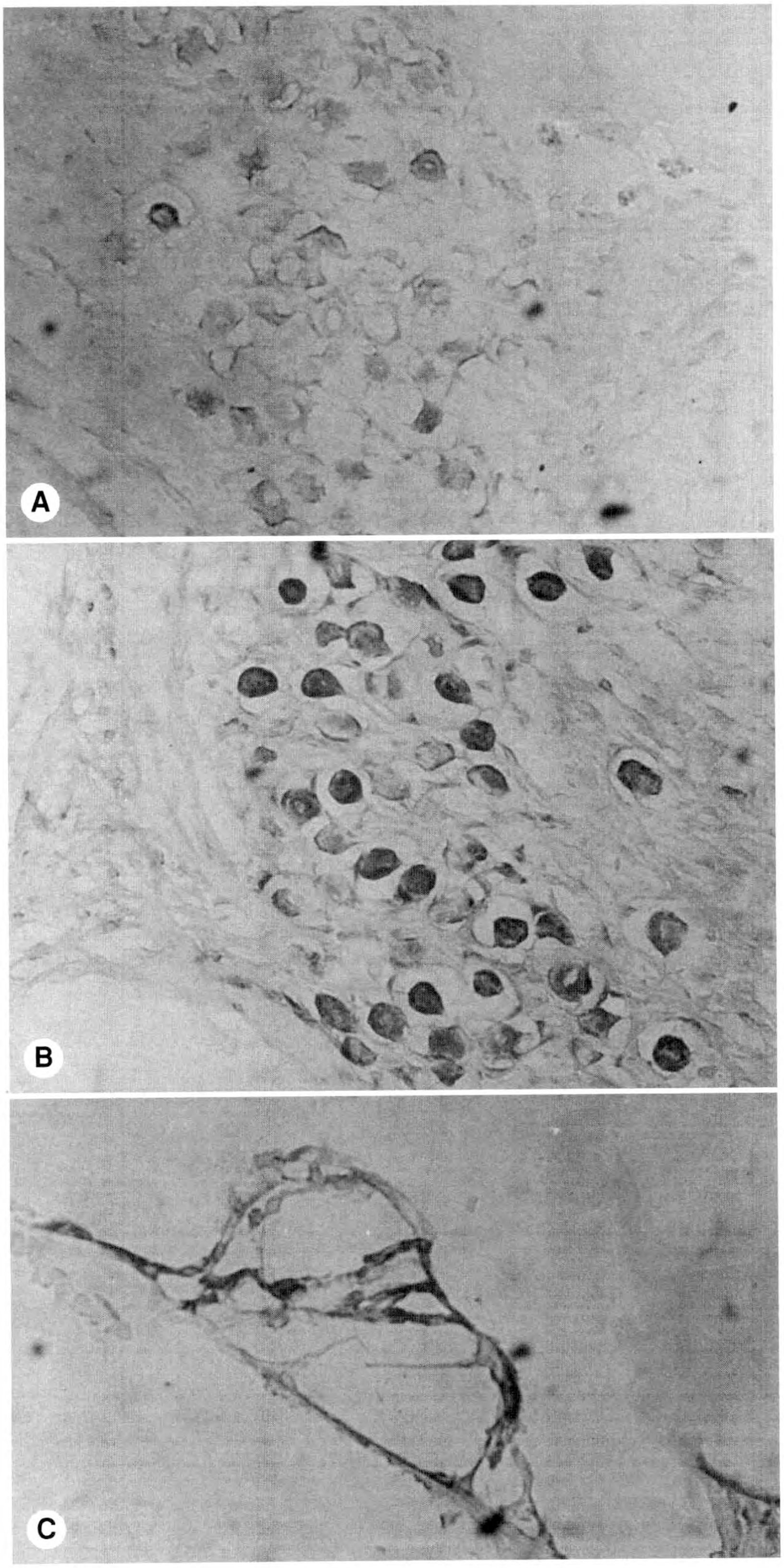

Fig 2. Expression of hsp70-like proteins (streptavidin-biotin-peroxidase method, original ×400). **A**) In cytoplasm of spiral ganglion cells of control animal with normal hearing. There is faint hsp70 immunostaining in cytoplasm. **B**) In cytoplasm of spiral ganglion cells of experimental animal with significant hearing loss. There is intense immunostaining in cytoplasm. **C**) In organ of Corti of experimental animal with significant hearing loss. There is staining of hair cells, pillar cells, and supporting cells.

are fundamental requirements for life, and consequently these molecules have remained highly conserved during evolution. Synthesis of the heat shock proteins, especially the 70 kd heat shock protein (hsp70), is readily induced by exposure to various forms of stress,[12] although its presence in a cell is not dependent upon stressful conditions. It mainly acts as a molecular chaperone involved in protein

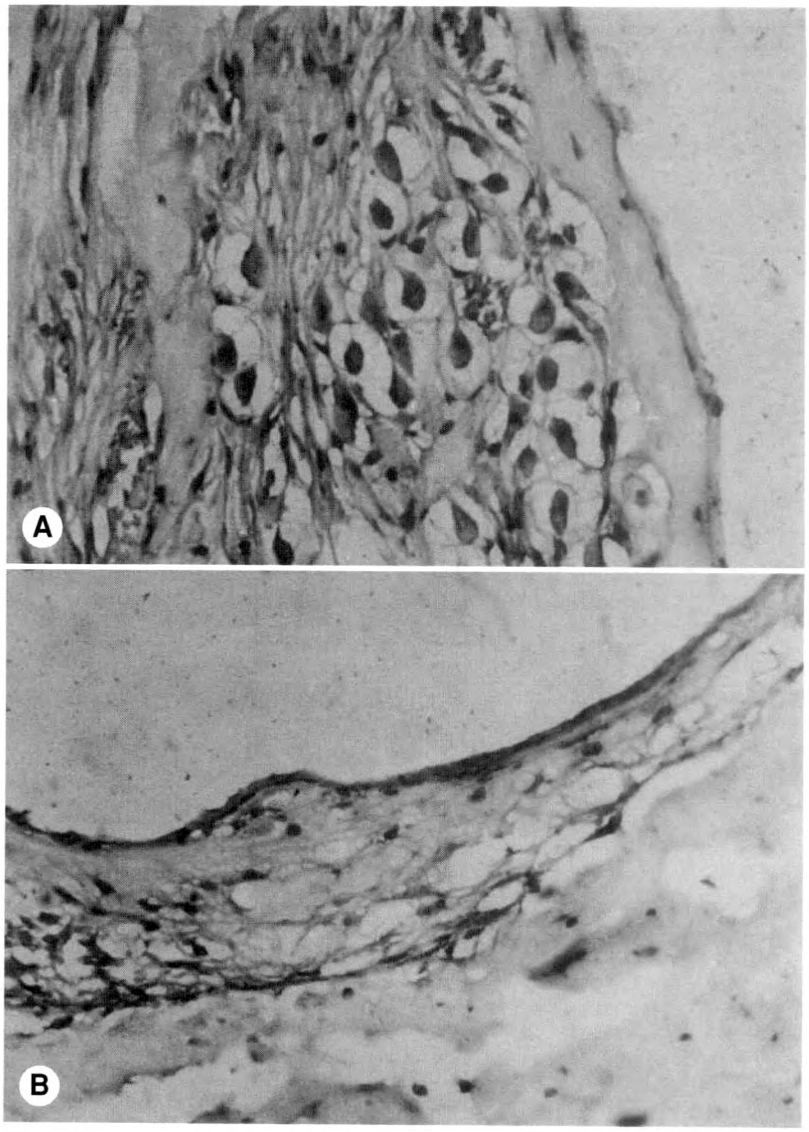

Fig 3. Expression of hsp70 messenger RNA in inner ears of experimental animals with significant hearing loss, detected by in situ hybridization (original ×400). **A**) Positive staining can be seen in cytoplasm of spiral ganglion cells. **B**) Strong hybridization signal can be seen in spiral ligament and stria vascularis.

synthesis and maturation in normal cells.[12] In a number of cell types, hsp70 is expressed at a very low level. A basic hsp70-like expression in the normal guinea pig cochlea was confirmed by the streptavidin-biotin-peroxidase technique in our study, and the immunolabeling of hsp70 was mainly seen in interdental cells and spiral ganglion cells. However, a negative result was obtained in the cochleas of the normal animals by in situ hybridization with the specific hsp70 probe. The present data support the conclusion that a baseline level of expression of other heat shock proteins that share common antigenic epitopes with hsp70 (hsp70-like proteins) lies in the normal guinea pig cochlea, and that there is no or very little expression of hsp70 itself in the normal inner ear.[6] The 68 kd protein that was found by Zou et al[6] in the guinea pig inner ear may differ from the 68 kd molecule identified in the bovine labyrinth by Harris and Sharp,[1] and may not be hsp70. The presence of hsp70-like proteins in the normal ear may be responsible for the occurrence of AIED that is induced by suppurative otitis media; in the period of middle ear infection, bacterial hsp70 could enter the inner ear through the round window membrane.[2] As we know, the endolymphatic sac has the capacity to mount immunologic responses in the inner ear; thus, we could easily speculate that enough bacterial hsp70 in the inner ear would induce the development of antibodies or lymphocytes sensitized to this protein. Because heat shock proteins are highly conserved from bacteria to humans, and the bacterial heat shock proteins are immunologically cross-reactive with their mammalian counterparts, those anti–bacterial hsp70 antibodies or activated lymphocytes could also react with the hsp70-like proteins expressed in the inner ear cells, and this immune response may damage the inner ear tissues.

In the present study, immunizing the animals with homologous CIEAg resulted in hearing deficit, and a hearing loss of 10 dB or more was found in 35.7% of the tested ears in the experimental group. The expression of hsp70 (perhaps also including hsp70-like molecules) and its messenger RNA was obviously increased in the cochleas with significant threshold shift (≥10 dB), as seen on immunohistochemical analysis and in situ hybridization. Moreover, we found that 67.9% of the cochleas from the experimental group showed perivascular mononuclear cellular infiltration in the modiolar vessels, and in an earlier work[3] from our laboratory, immunoglobulin deposition in the same vessels was seen in 65.6% of the experimental ears after guinea pigs had been systemically immunized with 8 intradermal injections of homologous CIEAg given at weekly intervals. All of these pathological changes are characteristic of an autoimmune reaction. Thus, we speculated that systemic immunization with CIEAg would induce a humoral and/or cellular immune response against the cochlea. The response would lead to an immune-related inner ear tissue injury and, ultimately, auditory dysfunction. The genes of heat shock proteins in the inner ear cells could also be activated by the stimulation of the immune response, so the synthesis of hsp70 would increase. It may be that hsp70 serves to protect inner ear cells and helps to limit the denaturation and aggregation of structurally and functionally important cellular proteins. In addition, hsp70 can also facilitate the repair and renaturing process of these proteins. We also observed that the expression of hsp70 in the cochleas without an obvious threshold shift (<10 dB) in the experimental group was similar to that in the controls, and this finding may be attributed to a weaker intensity of the inner ear immune response. Further studies are required to determine whether overproduced hsp70 could be involved in the development of AIED by inducing a local immune response against itself.

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IMAGING CASE STUDY OF THE MONTH

PEDIATRIC VIRTUAL BRONCHOSCOPY

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INTRODUCTION

The evaluation and diagnosis of congenital anomalies of the tracheobronchial tree often require a multidimensional approach including a thorough physical examination, endoscopy, and imaging studies. Virtual endoscopic techniques allow for both endoluminal and extraluminal evaluation of tracheobronchial compression. These techniques may facilitate diagnosis and guide decision-making regarding therapy, particularly in children with potential distal tracheobronchial obstruction.

CASE REPORT

A $1^1/_2$-year-old girl was evaluated for increasing difficulty with feeding. She was born full-term with no notable abnormalities. Her mother noted that since infancy the child had suffered intermittent coughing spells when feeding, but she denied any history of cyanotic spells or reflex apnea. Over the past several months, the symptoms had worsened to the point that the child was mildly stridorous while feeding, and the coughing with feeding had increased. She had been hospitalized within the past month with a diagnosis of croup.

On physical examination, the child was well developed and was breathing without distress. Her upper airway examination yielded unremarkable findings. A soft tissue radiograph of the neck was also nonrevealing. After discussion with the family, we performed bronchoscopy and esophagoscopy.

Rigid bronchoscopic evaluation revealed an apparent extrinsic compression of the right distal trachea just proximal to the right main stem bronchus (Fig 1). The findings of esophagoscopy were unremarkable. As the differential diagnosis at this point included vascular compression of the distal tracheobronchial tree, a spiral computed tomography (CT) scan was performed, and with the use of the GE Workstation software, virtual bronchoscopic images were generated. Specifically, a multislice helical CT scan of the chest was performed with a GE Lightspeed system (General Electric Medical Systems, Milwaukee, Wisconsin) using a rapid bolus intravenous contrast technique, 2.5-mm slice collimation, and 1.25-mm overlapped reconstructions. The data set was used to create 3-dimensional volume-rendered images of the aorta and a 3-dimensional virtual bronchoscopy image set (Figs 2 and 3). The diagnosis was a double aortic arch, and consultation with a cardiovascular surgeon was obtained for definitive repair.

DISCUSSION

The complete vascular ring anomaly, or double

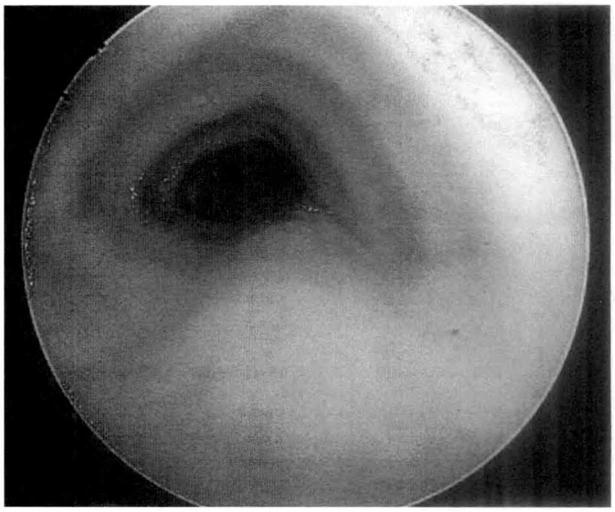

Fig 1. View from rigid bronchoscopy shows airway narrowing and right posterior impression from dominant right arch.

From the Departments of Otolaryngology (Hartnick, Chung, Myer) and Radiology (Emery), Children's Hospital Medical Center, Cincinnati Ohio.

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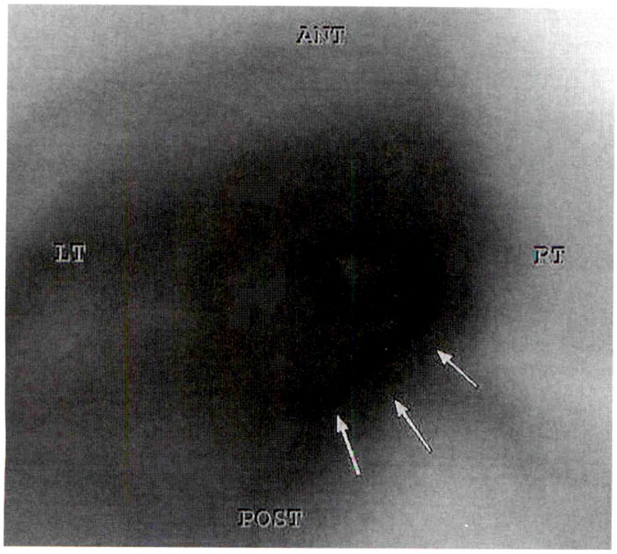

Fig 2. Three-dimensional view from virtual bronchoscopy shows airway narrowing and right posterior impression (arrows) from dominant right arch.

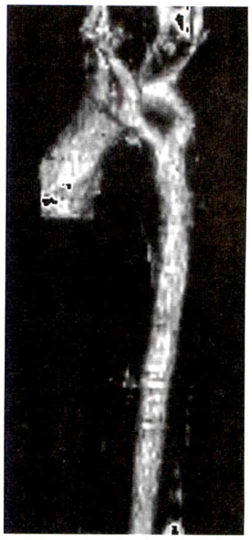

Fig 3. Three-dimensional volume-rendered image of aorta viewed from left posteriorly and superiorly demonstrates complete vascular ring created by smaller left arch and larger, dominant right arch.

aortic arch, is the second most common vascular anomaly causing tracheobronchial compression, following innominate artery compression of the trachea.[1,2] The symptom complex produced by a double aortic arch mimics those produced by other vascular anomalies that impinge upon both the airway and the esophagus. Upper respiratory tract infections can produce aggravation of the symptoms. Diagnosis has consisted of endoscopy coupled with either magnetic resonance imaging or spiral CT imaging with intravenous contrast material.[3] Treatment involves division of the nondominant arch.

Virtual endoscopy has been described both in the field of otolaryngology and in the fields of general surgery and neurosurgery since the early 1990s.[4-6] Applications involving the field of otolaryngology include evaluation of the paranasal sinuses before surgery[7] and evaluation of the upper airway for head and neck malignancy.[8,9] The role of the virtual endoscope as a surgical teaching aid has been explored in the fields of neurosurgery,[10] orthopedics,[11] and otolaryngology.[12]

There has been comparatively little written about the role of virtual endoscopy with reference to the pediatric population. The issue arises regarding its clinical utility. Evaluation of the child with an ill-defined reason for airway symptoms represents one potentially beneficial clinical application. Even with the use of modern spontaneous anesthetic techniques such as spontaneous ventilation, the child with a distal airway obstruction is at increased risk of airway compromise after administration of the anesthetic. High-kilovoltage radiographs of the airway are routinely acquired before laryngoscopy and bronchoscopy in order to diagnose a distal obstruction that might aggravate airway compromise when the child is anesthetized.[13] However, as was the situation in the case presented, there are potentially significant false-negative rates for high-kilovoltage films that vary according to the presenting disorder. Virtual endoscopy holds the potential to allow for evaluation of the airway in a relatively safe environment and to allow for treatment planning. The visual quality of virtual endoscopy and the costs and the current time burden necessary to produce the images prohibit its routine use; nevertheless, this technique may provide meaningful information for cases in which there is a diagnostic dilemma.

Virtual endoscopic techniques have been reported in several small studies in children that evaluated the ability to diagnose causes of extrinsic tracheobronchial compression.[3,14] Given the appropriate software package, these images are readily acquired. This case report suggests the potential clinical application of this technology. Future studies are needed to decide whether this technology represents an expensive novelty or a tool to provide meaningful information in the appropriate clinical scenario.

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LETTER TO THE EDITOR

To the Editor:

We read with great interest the article by Scott et al.[1] Among the lesions reported was that of a benign sessile endobronchial leiomyoma that developed in a 2-year-old girl with human immunodeficiency virus (HIV). She underwent successful endoscopic resection and presumably recovered without incident. While it was stated that her HIV infection was likely coincidental, there have been 2 other reported cases of histologically benign endobronchial leiomyomas in HIV-infected children.[2] Unlike the case reported by Scott et al, both children ultimately died of progressive obstructive pneumonitis, with 1 having failed partial endoscopic resection.

Both children, one 32 months and the other 9 years of age, presented with symptoms of bronchial obstruction similar to those described by Scott et al,[1] and as commonly occurs, these cases were initially misdiagnosed as gastroesophageal reflux disease and reactive airways disease with mucus plugging, respectively.[2] Chest radiographs taken at the time of presentation showed interstitial infiltrates in the first child and lobar atelectasis in the second. Receiving only comfort care because of advanced, progressive HIV encephalopathy, the first child eventually died of respiratory failure and necrotizing bronchopneumonia; lung cultures revealed cytomegalovirus, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. However, postmortem examination also revealed 4 clinically unsuspected endobronchial leiomyomas measuring between 0.5 and 1 cm in diameter in several lobes, all with near-total bronchial obstruction. Leiomyomas were also found in the spleen, liver, and left adrenal gland.

After a computed tomography scan of the chest showed severe narrowing of the left bronchus, the second child underwent bronchoscopy and was similarly found to have subtotal obstruction of the left main stem bronchus by a leiomyomatous lesion that was initially thought to represent granulation tissue by its appearance on endoscopy.[2] Despite broad-spectrum antibiotics and partial debulking of the lesion by endobronchial biopsy, this child went on to develop progressive air trapping and ultimately died within 4 months of presentation. As in the first case, postmortem examination revealed several radiographically inapparent, well-circumscribed leiomyomas in and around the bronchi bilaterally, measuring up to 1.5 cm in diameter. Associated abscesses were found in both lungs that grew *Aspergillus* spp and *Mycobacterium avium* complex. A leiomyomatous lesion was found in the spleen of this child, as well.

As demonstrated in these 2 cases, although histologically benign, endobronchial leiomyomas that develop in immunocompromised individuals may in fact behave in a malignant fashion, as clinically imperceptible lesions may develop not only multifocally in and around the airways, but in multiple organs, as well.[2] Furthermore, as reported in an adult with HIV, even when endoscopically resectable, such lesions may require repeated attempts because of their progressive nature,[3] not unlike the papillomas associated with juvenile-onset recurrent respiratory papillomatosis also described by Scott et al in their article.[1] It is of interest to note that as human papillomavirus types 6 and 11 are believed to be primarily responsible for recurrent respiratory papillomatosis,[4] evidence of latent infection with Epstein-Barr virus has also been found in benign leiomyomas that develop in the setting of immunosuppression such as HIV infection.[2] The finding of clonal Epstein-Barr virus DNA in these lesions further suggests a pathogenetic role.[5]

In conclusion, we agree that endobronchial tumors should always be suspected in children with atypical or chronic respiratory complaints[1] such as wheezing unresponsive to bronchodilator therapy, and atypical radiographic findings such as unilateral air trapping.[2] However, while benign lesions such as those associated with juvenile-onset recurrent respiratory papillomatosis may indeed prove recurrent and recalcitrant to available medical and surgical interventions and have increased morbidity and mortality rates,[1,4] histologically benign endobronchial leiomyomas that develop in children infected with HIV may similarly prove to be "malignant by location."[6] These children are particularly susceptible to the ravages of superimposed infection and the risks associated with endoscopic and/or surgical intervention.

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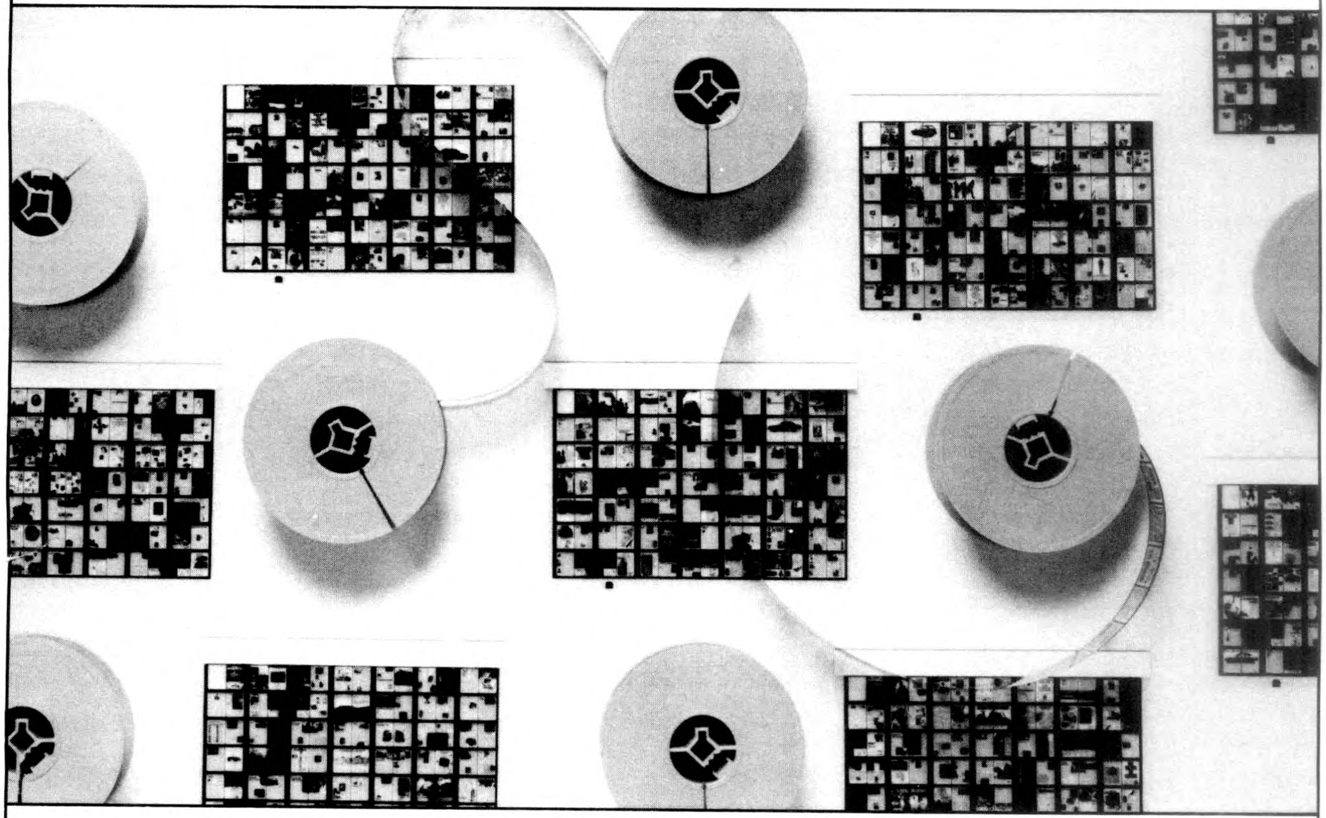

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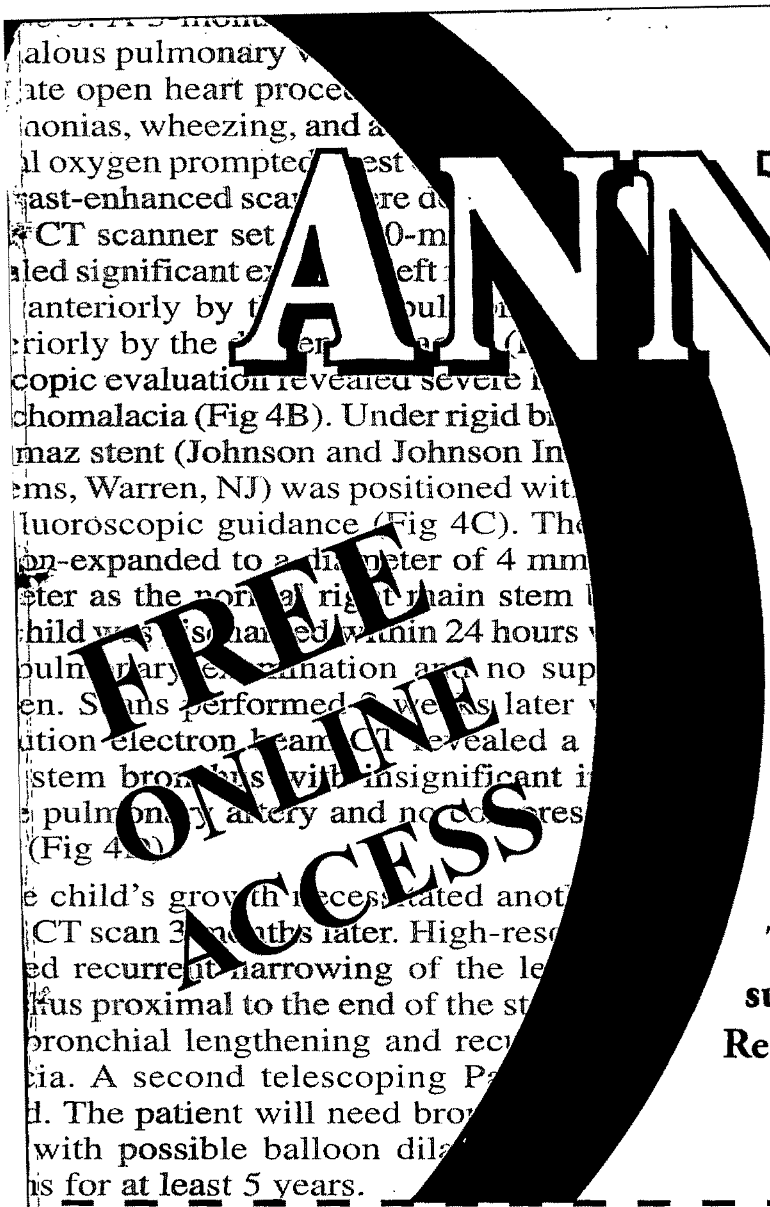

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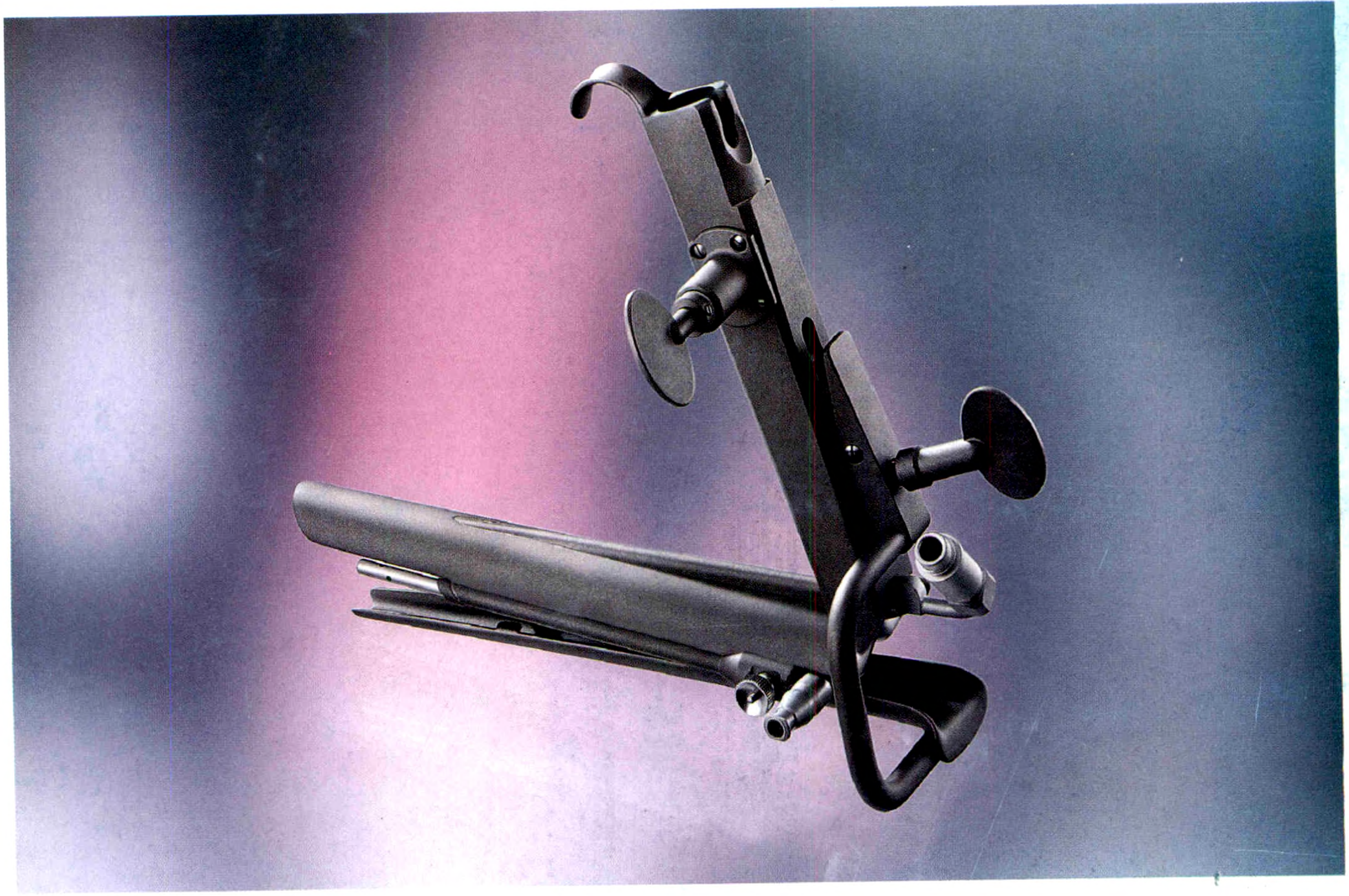

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RECENT ADVANCES IN OTITIS MEDIA
REPORT OF THE SEVENTH RESEARCH CONFERENCE

Editor
DAVID J. LIM, MD

Co-Editors
CHARLES D. BLUESTONE, MD
MARGARETHA L. CASSELBRANT, MD, PHD

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This Conference was sponsored by the Department of Pediatric Otolaryngology, Children's Hospital of Pittsburgh; the Center for Continuing Education in the Health Sciences, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; and the Gonda Department of Cell and Molecular Biology of the House Ear Institute. It was held in Fort Lauderdale, Florida, June 1-5, 1999, and was supported in part by grants from the National Institute on Deafness and Other Communication Disorders of the National Institutes of Health NIDCD R13 DC04169, the Deafness Research Foundation, and the House Ear Institute.

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INTRODUCTION

The Seventh Post-Symposium Research Conference was held at the Marriott Harbor Beach Resort in Fort Lauderdale, Florida, on June 5 and 6, 1999. The approximately 70 participants included otolaryngologists, pediatricians, audiologists, epidemiologists, microbiologists, immunologists, and cell and molecular biologists from the United States, Europe, Asia, and South America. These participants, as well as another 30 individuals unable to attend the Research Conference, contributed to this Research Conference Report.

The Post-Symposium Research Conferences were initiated to further capitalize upon the gathering of leading researchers in the field of otitis media that occurs for the International Symposium on Recent Advances in Otitis Media. Although Extraordinary Symposia have taken place between the International Symposia since 1985, Research Conferences have followed only the regularly scheduled Symposia and have occurred since the first International Symposium was held in 1975 in Columbus, Ohio.

The goals of the Research Conference are to 1) critically review the most recent advances and breakthroughs made during the 4 years since the previous Symposium; 2) develop a consensus on definitions and classification; 3) identify new research opportunities that have arisen because of technical advances; 4) promote research on otitis media among young scientists; 5) identify critical research questions; and 6) identify short-term and long-term research goals. The Research Conference Report was designed to be as critical, selective, and succinct as possible, so that the reader may use this handy research resource to follow recent advances and to understand the important questions that are challenging researchers.

The Research Conference Reports have been published by the *Annals of Otology, Rhinology & Laryngology* as supplements to ensure wide dissemination and distribution. Copies have been distributed to academic departments of otolaryngology, to academic departments of pediatric infectious diseases, and to all medical school libraries in the United States. Along with the Proceedings of the Otitis Media Symposium, the Research Conference Reports have been valuable resources for investigators interested in otitis media research and are especially helpful for those new to the field.

Research on otitis media has been advancing at a rather rapid pace, especially in recent years, in part because of the development of new technology and methodology. We believe that the Post-Symposium Research Conferences have also made significant contributions in stimulating otitis media research in many areas, including clinical and basic research.

Generating the Research Conference Report is not an easy task. A great deal of effort is required of the Panel Chairs, Co-Chairs, and Members. Because of the international composition of the Panels, there are many divergent philosophies and opinions, and we have tried to incorporate these differences in the Research Conference Report. We would like to thank the Panel Chairs and Co-Chairs for gathering information from the Panel Members and for generating these excellent reports, as well as all of the Panel Members who contributed to these reports.

On behalf of the Planning Committee of the Research Conference, we especially thank the National Institute on Deafness and Other Communication Disorders (NIDCD), the National Institutes of Health, the Deafness Research Foundation (DRF), the House Ear Institute, and the International Otitis Media Symposium Inc for their support for this Research Conference and for the partial funding of publication of the Report. We are especially grateful to Dr Jim Battey, Director of NIDCD; Dr Ralph Naunton, Director of Division of Human Communication; Dr Thane Cody, Medical Director of DRF; Mr Jim Boswell, CEO, House Ear Institute; and Dr Tom Johnson, Health Scientist Administrator, NIDCD, whose support for the Research Conference was critical.

We also thank the Center for Continuing Education in Health Sciences of the University of Pittsburgh Medical Center and Fran Arre of Forbes' Travel Meeting Management Division for providing logistical support for the Research Conference. Robbin Roberts, Colleen Young, Susan Early, Liz Hansen, and Dr Ali Andalibi from the Office of the Executive Vice President of Research at the House Ear Institute provided superb editorial assistance.

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SPECIAL REMARKS

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DIRECTOR, DIVISION OF EXTRAMURAL RESEARCH

NATIONAL INSTITUTE ON DEAFNESS AND OTHER COMMUNICATION DISORDERS

Otitis media continues to be an enormous problem worldwide. In the United States, health care costs exceed $5 billion per year, and myringotomy with tube insertion is the most frequently performed otolaryngological operation. In addition, children and their families carry a heavy burden of suffering, inconvenience, and unmeasured economic losses through travel costs and lost work time. We are still unsure of the impact of otitis media and its attendant fluctuating hearing loss on speech and language development and on cognitive and academic performance, but even small effects in these areas, multiplied by millions of children, are important. Too often overlooked are the delayed effects of childhood otitis media in adults: the National Study of Hearing in the 1980s found that more than 4% of the British adult population had middle ear disease with associated conductive hearing loss (which was about equally divided between otosclerosis and sequelae of otitis media). As significant as these problems are in Europe and the United States, otitis media is an even worse scourge in developing countries.

Basic and clinical research remains our best hope for controlling otitis media. Since 1975, the series of International Symposia on Recent Advances in Otitis Media and the Post-Symposium Research Conferences, of which this is the seventh, have brought together the best ideas, the best new knowledge, and — most important — the best people in the field. In that quarter of a century, our ability to probe and to begin understanding the genetic, molecular, and cellular phenomena underlying the clinical entity of otitis media has expanded in ways that would have been unimaginable in 1975. These successes are partly attributable to the recent and remarkable advances in basic biology, but we must also acknowledge the important role that meetings like these play in catalyzing intellectual reactions, facilitating communication, and forging scientific partnerships. Drs Lim, Bluestone, and Casselbrant and their Program Committee deserve our congratulations for their efforts.

The National Institute on Deafness and Other Communication Disorders is proud to have sponsored the Research Conference and looks forward to many years of fruitful collaboration with the otitis media research community.

PANEL REPORTS

1. DEFINITIONS, TERMINOLOGY, AND CLASSIFICATION OF OTITIS MEDIA

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INTRODUCTION

We are indebted to the late Ben H. Senturia for organizing a small task force of clinicians and scientists to meet with him in St Louis in 1978 to reach a consensus on the definitions, terminology, and classification of otitis media. The report of the task force was published in the proceedings of the Second International Symposium on Recent Advances in Otitis Media.[1]

Even though some lingering controversy still exists, there is now general consensus over the terminology and definitions used to describe the various stages of otitis media. It is important to reach agreement about these issues so that health care professionals can effectively communicate, both clinically and scientifically, with each other.

The 1978 task force did not include grading of acute otitis media and otitis media with effusion, related to severity, or a classification of the complications and sequelae of otitis media, and related conditions, in their deliberations. Many of the terms were defined before the advent of modern otology, which has afforded us the opportunity to examine patients with the operating microscope, both in the ambulatory setting and at the time of otologic surgery. Also, the recent availability of radiologic imaging technology has allowed us to visualize contents of the temporal bone and the intracranial cavity that were not accessible to the pioneers in otology.

There is a need for a group similar to the 1978 task force to reach consensus on a classification of the complications and sequelae, as the task force did in defining acute otitis media and otitis media with effusion. Also, there are no acceptable staging systems for these complications and sequelae. If available, such staging systems would enhance our ability to study the natural history of the diseases and to conduct and evaluate research in a more uniform manner from center to center and country to country, which, in turn, would result in more effective management of patients. (The grade of otitis media, and related diseases and disorders, relates to the severity of the condition, whereas a stage is a period or distinct phase in the course of otitis media or one of its complications or sequelae.)

To achieve evidence-based information in the future depends on development of a universally acceptable classification, as well as grading and staging systems, for the otogenic complications and sequelae.

The definitions of the most commonly used terms follow. Otitis media is an inflammation of the middle ear without reference to etiology or pathogenesis. Acute otitis media is the rapid onset of signs and symptoms, such as otalgia and fever, of acute infection within the middle ear. Otitis media with effusion is a chronic inflammation of the middle ear in which a collection of liquid is present in the middle ear space, but there is an absence of the signs and symptoms of acute infection. (No perforation of the tympanic membrane is present.) Middle ear effusion is a liquid in the middle ear, but the term does not designate etiology, pathogenesis, pathology, or duration. On visual examination of the fluid, an effusion may be 1) serous, with a thin, watery liquid; 2) mucoid, with a thick, viscid, mucus-like liquid; 3) purulent, with a pus-like liquid; or 4) a combination of these. An effusion can be the result of either acute otitis media or otitis media with effusion. The effusion can be of recent onset and acute, or more long-lasting, subacute, and chronic. Otorrhea is a discharge from the ear that may be from one or more of the following sites: external auditory canal, middle ear, mastoid, inner ear, or intracranial cavity.

Table 1 shows a proposed classification derived from our present knowledge of otitis media and its

TABLE 1. CLASSIFICATION OF OTITIS MEDIA

- 1. Otitis media
  - 1.1 Acute otitis media (AOM)
  - 1.2 Chronic otitis media (COM)
    - 1.2.1 With effusion (OME)
    - 1.2.2 Without effusion
    - 1.2.3 Acute exacerbation
- 2. Eustachian tube dysfunction
- 3. Intratemporal (extracranial) complications and sequelae
  - 3.1 Hearing loss
    - 3.1.1 Conductive
    - 3.1.2 Sensorineural
  - 3.2 Perforation of tympanic membrane
    - 3.2.1 Acute perforation
      - 3.2.1.1 without otitis media
      - 3.2.1.2 with otitis media (acute otitis media with perforation)
        - 3.2.1.2.2 with otorrhea
    - 3.2.2 Chronic perforation
      - 3.2.2.1 without otitis media
      - 3.2.2.2 with otitis media
        - 3.2.2.2.1 acute otitis media
          - 3.2.2.2.1.1 without otorrhea
          - 3.2.2.2.1.2 with otorrhea
        - 3.2.2.2.2 chronic otitis media (and mastoiditis; chronic suppurative otitis media)
          - 3.2.2.2.2.1 without otorrhea
          - 3.2.2.2.2.2 with otorrhea
  - 3.3 Mastoiditis
    - 3.3.1 Acute
      - 3.3.1.1 acute mastoiditis with otitis media
      - 3.3.1.2 acute mastoiditis with periosteitis
        - 3.3.1.3.1 without subperiosteal abscess
        - 3.3.1.3.2 with subperiosteal abscess
    - 3.3.2 Chronic
      - 3.3.2.1 without chronic otitis media
      - 3.3.2.2 with chronic otitis media
  - 3.4 Apical petrositis
    - 3.4.1 Acute
    - 3.4.2 Chronic
  - 3.5 Facial paralysis
    - 3.5.1 Acute
    - 3.5.2 Chronic
  - 3.6 Labyrinthitis
    - 3.6.1 Acute
      - 3.6.1.1 serous
        - 3.6.1.1.1 localized (circumscribed)
        - 3.6.1.1.2 generalized
      - 3.6.1.2 suppurative
        - 3.6.1.2.1 localized
        - 3.6.1.2.2 generalized
    - 3.6.2 Chronic
      - 3.6.2.1 Labyrinthine sclerosis
  - 3.7 Atelectasis of middle ear
    - 3.7.1 Localized
      - 3.7.1.1 without retraction pocket
      - 3.7.1.2 with retraction pocket
    - 3.7.2 Generalized
  - 3.8 Aural acquired cholesteatoma
    - 3.8.1 without infection
    - 3.8.2 with infection
      - 3.8.2.1 acute
        - 3.8.2.1.1 without otorrhea
        - 3.8.2.1.2 with otorrhea
        - 3.8.2.2.1 without otorrhea
        - 3.8.2.2.2 with otorrhea
  - 3.9 Cholesterol granuloma
  - 3.10 Ossicular discontinuity
  - 3.11 Adhesive otitis media
  - 3.12 Tympanosclerosis
  - 3.13 Ossicular fixation
  - 3.14 Infectious eczematoid dermatitis
    - 3.14.1 acute infectious eczematoid dermatitis
    - 3.14.2 chronic infectious eczematoid dermatitis
- 4. Intracranial complications
  - 4.1 Meningitis
  - 4.2 Extradural abscess
  - 4.3 Subdural empyema
  - 4.4 Encephalitis
  - 4.5 Brain abscess
  - 4.6 Dural sinus thrombosis
  - 4.7 Hydrocephalus

complications and sequelae. Terminology and definitions are described below according to this classification. Complications and sequelae of otitis media are classified into intratemporal (extracranial) complications and sequelae, which are those that occur within the temporal bone, and those that occur within the intracranial cavity (intracranial complications).

OTITIS MEDIA WITHOUT EFFUSION

There is a state of otitis media in which only inflammation of the middle ear mucous membrane and tympanic membrane will be present, with no evidence of a middle ear effusion. This state can occur during the early stages of otitis media, or during the stage of resolution, and can even be present as chronic disease. Myringitis may or may not be present.

ACUTE OTITIS MEDIA

The rapid and short onset of signs and symptoms of inflammation in the middle ear is characteristic of acute otitis media. Acute suppurative otitis media and purulent otitis media are synonyms still used by some, but are not currently recommended terms. One or more local or systemic signs are present: otalgia (or pulling of the ear in the young infant), otorrhea, fever, recent onset of irritability, anorexia, vomiting, or diarrhea. The tympanic membrane is full or bulging, is opaque, and has limited or no mobility to pneumatic otoscopy, all of which are indicative of middle ear effusion. Erythema of the eardrum is an inconsistent finding. The acute onset of ear pain, fever, and discharge (otorrhea) through a perforation of the tympanic membrane (or tympanotomy tube) would also be evidence of acute otitis media, ie, acute otitis media with perforation, but can be considered today as a complication (see below). When conducting and reporting research on acute otitis media, the investigator should precisely define acute otitis media, as well as describe the method of diagnosis; tympanocentesis is the most accurate method of diagnosis.

There may be some advantage in grading the severity of acute otitis media, since the outcome of treatment, or no treatment, may vary. Acute otitis media has been graded according to the severity of the disease. Kaleida et al[2] graded acute otitis media in infants and children who were entered into a clinical trial of the efficacy of antibiotics or myringotomy or both in subjects who had acute "severe" otitis media, whereas in subjects who were judged to have acute "nonsevere" otitis media, the efficacy of antibiotics was compared to that of placebo. Enrollment criteria were based on an otalgia scoring system that took into account estimated parental anxiety and reliability, and assigned 1, 3, or 12 points, respectively, for each hour of otalgia or apparent discomfort (in infants, ear pulling or irritability) rated as mild, moderate, or severe. An episode of acute otitis media was classified as "severe" if the subject's temperature had reached 39°C orally or 39.5°C rectally within the 24-hour period before presentation, or if the child attained an otalgia point score of at least 12 points. Episodes of acute otitis media meeting neither of these criteria were classified as "nonsevere."

OTITIS MEDIA WITH EFFUSION

Relatively asymptomatic effusion that develops in the middle ear has many synonyms, such as secretory, nonsuppurative, or serous otitis media, but the most acceptable term is otitis media with effusion. Because the effusion may be serous (transudate), the term secretory may not be correct in all cases. Likewise, the term nonsuppurative may not be correct, as asymptomatic middle ear effusion often contains bacteria and may even be purulent.[3-5] Pneumatic otoscopy will frequently reveal a retracted or convex tympanic membrane in which the mobility is impaired. But fullness or even bulging may be visualized in some patients. Also, an air-fluid level, bubbles, or both may be observed through a translucent tympanic membrane. The most important distinction between this type of disease and acute otitis media is that the signs and symptoms of acute infection (eg, otalgia and fever) are lacking in otitis media with effusion, but hearing loss is usually present in both conditions. As with acute otitis media, in conducting and reporting research on otitis media with effusion, the disease under study should be precisely defined.

Similar to a grading system for acute otitis media, there may be an advantage to grading otitis media with effusion according to severity, since the natural history or the effect of treatment or both may vary. One system proposes treatment of young children who have otitis media with effusion that is associated with bilateral hearing loss of 20 dB hearing threshold or worse in the better-hearing ear, whereas children who have better hearing or have a unilateral effusion or both are candidates for observation.[6] An alternative grading method is to use a tympanometric pattern classification. For example, a flat pattern would be considered more severe than one with any degree of gradient. Still another system could be grading according to otoscopic appearance. For example, a tympanic membrane that is completely opaque and immobile to pneumatic otoscopy would indicate more severe disease than a tympanic membrane that is translucent and mobile to pneumatic otoscopy in which bubbles or an air-fluid level or both can be seen through the eardrum. The distinction between mild, moderate, and severe is based on one or more of the following: otoscopic appearance, tympanometric patterns, or hearing thresholds. The duration (not the severity) of the effusion can be acute (less than 3 weeks), subacute (3 weeks to 3 months), or chronic (longer than 3 months).

EUSTACHIAN TUBE DYSFUNCTION

Eustachian tube dysfunction is a middle ear disorder that can have symptoms similar to those of otitis media, such as hearing loss, otalgia, and tinnitus, but no middle ear effusion is present. The dysfunction may be related to a eustachian tube that is too closed (ie, obstructed) or too open (ie, patulous); the latter condition is most frequently associated with symptoms of autophony.

The severity of this condition can be graded according to 1) mild, moderate, or severe symptoms, ie, the frequency, duration, and severity of symptoms; 2) the degree of disability caused by the symptoms, such as tinnitus, otalgia, autophony, dysequilibrium or vertigo, and hearing loss; or 3) duration, whether acute, subacute, or chronic (similar to duration as recommended above for grading otitis media with effusion).

INTRATEMPORAL (EXTRACRANIAL) COMPLICATIONS AND SEQUELAE OF OTITIS MEDIA

The following intratemporal complications and sequelae are classified into complications first and then sequelae, but some may be both a complication and a sequela, such as hearing loss. A complication of otitis media is another disease or disorder that is concurrent with the otitis media, whereas a sequela of otitis media is a disease or disorder that follows, is a consequence of, or is caused by otitis media. Also, a complication or sequela may also cause another complication or sequela; eg, a cholesteatoma may cause facial paralysis.

Many of the complications and sequelae of otitis media can also be iatrogenic, such as those that can follow tympanostomy tube insertion, tympanoplasty, or tympanomastoidectomy. These can include tympanosclerosis, adhesive otitis media, ossicular discontinuity or fixation, or cholesteatoma, all of which in turn may cause conductive hearing loss.

Hearing Loss. The most common complication and sequela of otitis media, hearing loss, can be either conductive or sensorineural, or both. When it is conductive, it may be either transient or permanent, but when it is sensorineural, it is usually permanent.

Fluctuating or persistent loss of hearing is present in most children who have middle ear effusion due to acute otitis media or otitis media with effusion. The hearing loss can be either mild or moderate, with the maximum loss being no greater than 60 dB. However, the loss is usually mild to moderate, in the range of 15 to 40 dB. When due to otitis media with effusion, there is an average loss of 27 dB.[7] Hearing loss associated with middle ear effusion is usually transient, but permanent conductive hearing loss can occur as a result of recurrent acute or chronic inflammation due to either adhesive otitis media or ossicular discontinuity or fixation. Also, negative pressure in the ear, in the absence of middle ear effusion, can be a cause of conductive hearing loss.[8] Patients who have eustachian tube dysfunction and intermittent or persistent high negative pressure can have an associated conductive hearing impairment.

Although the subject is still debated, hearing loss caused by chronic and recurrent middle ear effusions may be associated with delay or impairment of speech, language, and cognition in young children, which may or may not affect later school performance.[9,10]

Otitis media as a cause of sensorineural hearing loss can be in the form of acute otitis media, otitis media with effusion, or another complication or sequela of otitis media, such as chronic suppurative otitis media. The sensorineural hearing loss can be mild, moderate, severe, or profound.

Perforation of Tympanic Membrane. A perforation of the tympanic membrane can be either acute or chronic. Otitis media may or may not be present, and when otitis media is present, otorrhea may or not be present. Any classification of perforation should include the site, extent, and duration of the perforation, but no classification exists that has received widespread acceptance.

A reasonable classification related to site, extent, and duration is the following. The site may be 1) the pars tensa (anterosuperior, anteroinferior, posterosuperior, or posteroinferior quadrant) or 2) the pars flaccida. The extent may be 1) limited to 1 quadrant of the pars tensa (less than 25%); 2) may involve 2 or more quadrants, or 3) may be total, ie, all 4 quadrants. The duration may be 1) acute or 2) chronic.

Perforation of the tympanic membrane, when acute, is most commonly associated with otitis media (with or without otorrhea), but may also occur without otitis media. The presence of otorrhea is a manifestation of otitis media when there is a perforation. Most commonly, acute perforation without otitis media occurs after acute perforation *with* otitis media; ie, the middle ear inflammation is resolved, but the perforation persists.

One of the most common complications of acute otitis media is perforation of the tympanic membrane in which there is acute drainage (otorrhea) through the defect (ie, acute otitis media with perforation). Also, an acute perforation can be present in which there is otitis media but there is no evidence of otorrhea. Acute perforation can also occur as a complication of chronic otitis media with effusion, as has been reported in Australian Aborigines.[11]

When an attack of acute otitis media is complicated by a perforation (otorrhea is usually present), 1 of 4 outcomes is possible: 1) the acute otitis media resolves, and the tympanic membrane defect heals; 2) the acute otitis media resolves, but the perforation becomes chronic; 3) the perforation and otitis media persist into the chronic stage (ie, chronic suppurative otitis media); or 4) a suppurative complication of otitis media develops.

Chronic perforation occurs when an acute perforation fails to heal after 3 months or longer. A chronic perforation of the tympanic membrane can be present without otitis media or with otitis media; the latter condition may or may not be associated with otorrhea. Some clinicians consider a chronic perforation that is without otorrhea as "inactive" chronic suppurative otitis media, and a chronic perforation that is associated with otorrhea as "active" chronic suppurative otitis media.[12] But not only is this classification confusing, it is inappropriate in some cases, such as when there is a chronic perforation and the middle ear never becomes infected. Inclusion of chronic perforations, irrespective of the status of the middle ear, under the term chronic otitis media is not desirable;

in addition, the term chronic otitis media is confusing and potentially misleading and should be avoided (see below).

A chronic perforation that is not associated with either acute otitis media or chronic suppurative otitis media usually does not heal spontaneously. But the middle ear is susceptible to acute otitis media — and subsequently, chronic suppurative otitis media — when a perforation is present.

When a chronic perforation is associated with otitis media, the middle ear (and mastoid) infection can be either acute or chronic. The mastoid gas cell system is invariably involved when the inflammatory process is chronic. When either acute otitis media or chronic suppurative otitis media is present, otorrhea may or may not be evident.

Mastoiditis. Mastoiditis may or may not be a suppurative complication of otitis media, since both acute otitis media and otitis media with effusion can also involve the mastoid. Mastoiditis may be acute, subacute, or chronic. The following classification of the stages of this suppurative complication is proposed.

Acute mastoiditis can be staged as follows: 1) acute mastoiditis–otitis media, 2) acute mastoiditis with periosteitis, or 3) acute mastoid osteitis.

Acute mastoiditis may be a natural extension and part of the pathological process of acute middle ear infection. Most patients with acute otitis media probably have extension of the middle ear disease into the mastoid gas cell system, but this stage of acute mastoiditis is not strictly a complication of otitis media. Nevertheless, this stage can be misinterpreted as a complication of otitis media, especially when computed tomography (CT) scans are obtained for other reasons, eg, after head trauma or during an episode of otitis media. However, no specific signs or symptoms of mastoid infection, such as protrusion of the pinna, postauricular swelling, tenderness, pain, or erythema, are present in this most common type of mastoiditis. This stage of mastoiditis can either resolve (most common) or progress into a true complication of otitis media, ie, acute mastoiditis with periosteitis, which in turn can progress into acute mastoid osteitis.

When infection within the mastoid spreads to the periosteum covering the mastoid process, periosteitis can develop. This stage of acute mastoiditis should not be confused with the presence of a subperiosteal abscess. Acute mastoiditis with periosteitis is characterized by erythema, mild swelling, and tenderness in the postauricular area; the pinna may or may not be displaced inferiorly and anteriorly, with loss of the postauricular crease.

Acute mastoid osteitis has also been termed acute "coalescent" mastoiditis or acute surgical mastoiditis, but in reality the pathological process is osteitis. When infection within the mastoid gas cell system progresses, rarefying osteitis can cause destruction of the bony trabeculae that separate the mastoid cells. The postauricular area is usually involved, but mastoid osteitis can occur with no evidence of postauricular involvement. The signs and symptoms are similar to those described above for acute mastoiditis with periosteitis; a subperiosteal abscess may or may not be present.

Although relatively uncommon, if an acute middle ear and mastoid infection fails to totally resolve within the usual 10 to 14 days, subacute mastoiditis may develop. This stage has also been termed "masked mastoiditis." The classic signs and symptoms of acute mastoiditis are usually absent, such as pinna displacement, postauricular erythema, or subperiosteal abscess, but otalgia — including postauricular pain — and fever may be present. The diagnosis is made by CT scan. In this stage, the infection in the mastoid can progress into another intratemporal complication, or even an intracranial complication.

A chronic infection of the mastoid is usually due to chronic suppurative otitis media; a chronic perforation of the tympanic membrane is present. But chronic mastoiditis can occur in the absence of chronic suppurative otitis media. Patients who have relatively asymptomatic chronic otitis media with effusion frequently have some or all of the mastoid gas cell system also involved in the chronic disease process. This finding is commonly visualized on CT scans of the temporal bones. Also, chronic infection can be present in the mastoid, even in the absence of middle ear disease, because of obstruction of the aditus ad antrum: the otitis media resolved, but the disease in the mastoid did not. Symptoms can include low-grade fever and chronic otalgia and tenderness over the mastoid process.

Apical Petrositis. Infection from the middle ear and mastoid gas cells can spread into the petrosal gas cells. This disorder is called apical petrositis; it is also termed petrous apicitis or petrositis. This suppurative complication can be either acute or chronic, and can be due to either acute otitis media or chronic ear disease. When the infection is chronic, it is usually due to chronic suppurative otitis media, cholesteatoma or both.

Facial Paralysis. When facial paralysis is caused by otitis media — or one of its complications or sequelae — it may be either acute or chronic, and may

TABLE 2. HOUSE-BRACKMANN FACIAL NERVE GRADING SYSTEM[14]

Grade	*Description*	*Characteristics*
I	Normal	Normal facial function in all areas
II	Mild dysfunction	Slight weakness noticeable on close inspection; may have very slight synkinesis Normal symmetry and tone at rest Motion Forehead: moderate to good function Eye: complete closure with minimum effort Mouth: slight asymmetry
III	Moderate dysfunction	Obvious but not disfiguring difference between two sides; noticeable but not severe synkinesis, contracture, or hemifacial spasm Normal symmetry and tone at rest Motion Forehead: slight to moderate movement Eye: complete closure with effort Mouth: slightly weak with maximum effort
IV	Moderately severe dysfunction	Obvious weakness or disfiguring asymmetry Normal symmetry and tone at rest Motion Forehead: none Eye: incomplete closure Mouth: asymmetric with maximum effort
V	Severe dysfunction	Only barely perceptible motion Asymmetry at rest Motion Forehead: none Eye: incomplete closure Mouth: slight movement
VI	Total paralysis	No movement

be due to acute otitis media or chronic middle ear and mastoid disease such as cholesteatoma, chronic suppurative otitis media, or both.[13] The grading system of the degree of injury to the face proposed by House and Brackmann[14] is generally accepted, and correlates with recovery (Table 2). But this grading system does not necessarily apply to acute facial paralysis that is a complication of otitis media or its complications (eg, cholesteatoma) or is the result of surgery.

Labyrinthitis. When infection from the middle ear, mastoid gas cells, or both spreads into the cochlear and vestibular apparatus, the complication is termed labyrinthitis. The classification proposed by Schuknecht is appropriate, in which labyrinthitis is either serous labyrinthitis (also termed toxic labyrinthitis) or suppurative labyrinthitis.[15] Labyrinthitis may also be due to meningitis, which in turn may or may not be a complication of otitis media. Serous or suppurative labyrinthitis may be either acute or chronic, and either circumscribed or generalized. The end stage of chronic labyrinthitis is termed labyrinthine sclerosis.

Atelectasis of Middle Ear. Atelectasis of the middle ear is a sequela of otitis media, eustachian tube dysfunction, or both. Retraction or collapse of the tympanic membrane is characteristic of the condition. Collapse implies passivity (high negative middle ear pressure is absent), whereas retraction implies active pulling inward of the tympanic membrane, usually from negative middle ear pressure, which in turn is due to eustachian tube dysfunction. Middle ear effusion is usually absent when atelectasis is present. It may be acute or chronic; localized (with or without a retraction pocket) or generalized; and mild, moderate, or severe.

A classification of atelectasis has been proposed by Sadé that includes 4 stages in which the tympanic membrane is slightly retracted (stage 1), retracted onto the incus (stage 2), retracted onto the promontory (stage 3), or adherent in the sinus tympani, with accumulation of keratin, ie, cholesteatoma (stage 4).[16] But this system does not provide mutually exclusive staging, or include all anatomic sites or duration.

Atelectasis can be classified, graded, and staged in relation to extent and duration as follows.

A localized area of atelectasis of the tympanic membrane may or may not be a retraction pocket, since the depth of the retraction may be mild, moderate, or severe. When localized (with or without a retraction pocket), it may be in 1 of the 4 quadrants of the pars tensa (ie, anterosuperior, anteroinferior,

TABLE 3. STAGING OF RETRACTION POCKETS

Stage					
Acute	*Chronic*	*Approximates*	*Adherent*	*Expands With Pressure*	*Extent Visualized and Self-Cleansing*
1A	1C	No	No	Yes	Yes
2A	2C	Yes	No	Yes	Yes
3A	3C	Yes	Yes	No	Yes
4A	4C	Yes	Yes	No	No

Site(s) on tympanic membrane should also be designated.

posterosuperior, posteroinferior), in the pars flaccida, or in 2 or more of these anatomic sites. It is due to recurrent or chronic, moderate to severe underpressures in the middle ear, which are in turn due to eustachian tube dysfunction. In localized atelectasis without a retraction pocket, 1 or more quadrants of the pars tensa is atelectatic, but not all quadrants. The atelectasis can be mild, moderate, or severe, and acute or chronic. When it is severe, a retraction pocket is usually present.

A retraction pocket is characterized by a localized area of atelectasis of the tympanic membrane in which there is indrawing of the membrane forming borders (ie, edge or margin), most frequently at the site of an osseous anatomic structure (eg, notch of Rivinus or scutum) or the malleus. A retraction pocket can be in 1 or more of the 4 quadrants of the pars tensa or in the pars flaccida, and may be acute or chronic, and reversible or irreversible.

A classification of a posterosuperior retraction pocket has been proposed by Sadé[16] in which the pocket is slightly retracted and self-cleansing (stage 1), deeper and needing cleansing (stage 2), deeper still and partly hidden and requiring excision (stage 3), or so deep the pocket can only be removed by exposing the scutum and the rest of the framework (ie, retraction pocket cholesteatoma; stage 4).[16] This staging system is helpful, but does not include duration or the presence or absence of adhesive changes — which relate to reversibility — or include other sites.

If persistent and progressive, a retraction pocket can lead to sequelae commonly attributed to otitis media, such as hearing loss, ossicular chain discontinuity, and cholesteatoma.

Staging divides retraction pockets into retraction pockets that are acute (less than 3 months' duration) and those that are chronic (3 months or longer in duration; Table 3). Key factors that affect the progression of the stages of a retraction pocket from stage 1 to stage 4 are as follows. First is the relationship to middle ear structures: whether it approximates (touches) or is adherent to (ie, adhesive otitis media) 1 or more ossicles (ie, incus, incudostapedial joint, stapes, head of malleus, incudomalleal joint), or other middle ear structures, such as the promontory of the cochlea. Also considered is whether it expands with pressure, ie, whether the entire pocket easily expands to the normal position when negative pressure is applied with a pneumatic otoscope, or with the Bruening otoscope with a nonmagnifying lens under the otomicroscope, or with positive pressure when the patient is anesthetized with nitrous oxide. Third, one considers the extent to which the pocket is visualized, ie, whether the entire pocket is visualized, or whether parts are not seen even after applied pressure, because the pocket extends beyond the visible portion of the middle ear space (sinus tympani, facial recess, epitympanum) or medial to other parts of the tympanic membrane. Finally, one considers whether the pocket is self-cleansing and free of infection, ie, whether epithelial debris, crusting, or purulent material is present within the pocket.

A proposed staging system that takes into account the key factors given above is as follows. Stage 1A is an acute, mild retraction pocket, in which the membrane of the pocket neither approximates nor is adherent to any middle ear structure, the pocket expands with pressure, the entire contents of the pocket are readily visible, and the pocket is self-cleansing. Stage 1C, a chronic, mild retraction pocket, is the same as stage 1A, but is chronic. Stage 2A, an acute, moderate retraction pocket, has a membrane that is applied to 1 or more middle ear structures but is not adherent, expands with pressure, has an extent that can be visualized, and is self-cleansing without infection. Stage 2C, a chronic, moderate retraction pocket, is the same as stage 2A, but chronic. In stage 3A, an acute, severe retraction pocket, the tympanic membrane is adherent to 1 or more middle ear structures, the extent is visualized and is without infection, and the pocket cannot be expanded with pressure. Stage 3C, a chronic, severe retraction pocket, is the same as stage 3A, but chronic. Stage 4A, an acute retraction pocket–cholesteatoma, has a tympanic membrane that is adherent to 1 or more middle ear structures, and the extent cannot be visualized, or the pocket is not self-cleansing or free of infection. Stage 4C, a chronic retraction pocket–cholesteatoma, is the same as stage 4A, but chronic.

Acute (duration less than 3 months) and chronic

(3 months or longer) atelectasis that involves all 4 quadrants of the pars tensa, with or without involvement of the pars flaccida, can be staged as follows. Stage 1A is acute, generalized, mild atelectasis with the middle ear aerated. Stage 1C, chronic, generalized, mild atelectasis, is the same as stage 1A, but chronic. Stage 2A, acute, generalized, severe atelectasis, has a middle ear that is not aerated; ie, there is no apparent middle ear space. Stage 2C, chronic, generalized, severe atelectasis, is the same as stage 2A, but chronic.

Aural Acquired Cholesteatoma. When keratinizing stratified squamous epithelium accumulates in the middle ear or other pneumatized portions of the temporal bone, the disease is termed cholesteatoma; the term aural distinguishes this type of cholesteatoma from a similar pathological entity that occurs outside the temporal bone. "Acquired" distinguishes this type of cholesteatoma as a sequela of otitis media or related conditions (eg, retraction pocket of the tympanic membrane) from aural congenital cholesteatomas. Even though the term is a misnomer — keratoma is more consistent with the pathology — cholesteatoma is in common usage and thus accepted.[17] Even though cholesteatoma can be classified into congenital or acquired — the latter being further subclassified into a sequela of otitis media (and certain related conditions), or due to implantation, which may be due to trauma, or iatrogenic — otitis media may be involved in the pathogenesis of congenital cholesteatoma. Classically, congenital cholesteatoma develops as a congenital rest of epithelial tissue within the temporal bone, in the absence of a defect in the tympanic membrane. In contrast to this strict definition, an acquired aural cholesteatoma may develop from a retraction pocket in the pars tensa or pars flaccida (see above), migration of epithelium through a preexisting defect (eg, perforation) of the tympanic membrane, or, more rarely, metaplasia of the middle ear–mastoid mucous membrane. A cholesteatoma may involve the middle ear, mastoid, or both, invade the labyrinth, or even extend beyond the temporal bone.

Cholesteatoma may or may not be associated with otitis media (and mastoid), but when otitis media is present, the infection may be either acute or chronic, and otorrhea may or may not be present. A cholesteatoma can be a cyst-like structure with no signs of infection. A cholesteatoma that is present in association with chronic inflammation of the middle ear (and mastoid) would be defined as cholesteatoma with chronic suppurative otitis media. Thus, cholesteatoma may or may not be associated with chronic suppurative otitis media. It is inappropriate to include cholesteatoma in which there is no associated infection, such as chronic suppurative otitis media, under the term chronic otitis media.

Cholesteatoma can be staged as follows, according to the presence or absence of infection and, if present, the duration of the otitis media.

Cholesteatoma that is not associated with infection, either within the cholesteatoma itself or in any other portion of the middle ear cleft, can be further classified as to its site and extent. When cholesteatoma is associated with infection, it can be either acute infection (with or without otorrhea) or chronic suppurative otitis media.

The staging is as follows. In stage 1, cholesteatoma is confined to the middle ear (hypotympanum, mesotympanum, epitympanum), without erosion of the ossicular chain. Stage 2 is the same as stage 1, but erosion of 1 or more ossicles is present. In stage 3, the middle ear and mastoid gas cell system are involved without erosion of ossicles. Stage 4 is the same as stage 3, but erosion of 1 or more ossicles is present. In stage 5, extensive cholesteatoma of the middle ear, mastoid, and other portions of the temporal bone is present, the extent of which is not totally accessible to surgical removal (eg, medial to labyrinth), and 1 or more ossicles are involved; fistula of the labyrinth may or may not be present. Stage 6 is the same as stage 5, but the cholesteatoma extends beyond the temporal bone.

Cholesterol Granuloma. Cholesterol granuloma is a relatively uncommon sequela of otitis media. It has often been termed "idiopathic hemotympanum," but this term is a misnomer, since there is no evidence of blood in the middle ear.[18] Staging is related the site and extent of the cholesterol granuloma. In stage 1, the cholesterol granuloma is localized to 1 portion of the mastoid gas cell system or middle ear; in stage 2, the cholesterol granuloma involves the entire middle ear cleft.

Ossicular Discontinuity. Ossicular discontinuity, as a sequela of otitis media and certain related conditions, is the result of rarefying osteitis caused by the inflammation, but a retraction pocket or cholesteatoma can also cause resorption of ossicles. A classification of ossicular discontinuity is related to the site of pathology: 1) stapes crura, 2) incudostapedial joint, 3) incus, 4) incudomalleal joint, and 5) malleus.

Adhesive Otitis Media. Adhesive otitis media is a result of healing following chronic inflammation of the middle ear and mastoid. The mucous membrane is thickened by proliferation of fibrous tissue, which frequently impairs movement of the ossicles, resulting in conductive hearing loss. The pathological process is a proliferation of fibrous tissue within the middle ear and mastoid termed fibrous sclerosis.[19] When there are cystic spaces present, it is called fibrocystic sclerosis, and when there is new bone growth in the mastoid, it is termed fibro-osseous sclerosis.

The staging of adhesive otitis media is as follows: In stage 1, the adhesive otitis media is within the middle ear or mastoid, or both, and there is no functional deficit secondary to the adhesive changes, ie, hearing loss; the middle ear remains aerated. In stage 2, the adhesive otitis media is within the middle ear (with or without mastoid involvement) and there is mild hearing loss secondary to the adhesive pathology, such as involvement of the ossicular chain (fixation or discontinuity, or both; see Ossicular Discontinuity and Ossicular Fixation), limitation of tympanic membrane compliance, or both, but the middle ear remains aerated. Stage 3 is similar to stage 2, but there is maximum conductive hearing loss (secondary to ossicular pathology) and the absence of a middle ear space, both of which are due to the extensive adhesive otitis media.

Tympanosclerosis. Tympanosclerosis is characterized by the presence of whitish plaques in the tympanic membrane and nodular deposits in the submucosal layers of the middle ear. The pathological process in the tympanic membrane occurs in the lamina propria, whereas within the middle ear, the basement membrane is affected. In both sites, there is hyalinization followed by deposition of calcium and phosphate crystals. Conductive hearing loss may occur if the ossicles become embedded in the deposits. Conductive hearing loss secondary to tympanosclerosis that involves only the tympanic membrane is rare, even though scarring of the eardrum at the site of tympanostomy tube insertion is common.[20,21] Tympanosclerosis is usually a sequela of chronic middle ear disease (chronic otitis media with effusion or chronic suppurative otitis media), but is also associated with trauma, such as that caused by tympanostomy tube insertion.

The staging of tympanosclerosis is as follows. In stage 1, the tympanosclerosis is limited to the tympanic membrane (ie, little or no involvement of the middle ear), and the hearing is unaffected; the term myringosclerosis is also acceptable. This stage can be further categorized into the following subclasses: tympanosclerosis limited to 1 quadrant of the pars tensa (stage 1-1); tympanosclerosis limited to 2 or more quadrants, but no total involvement of the tympanic membrane (stage 1-2); and tympanosclerosis that totally involve the tympanic membrane (stage 1-3). Stage 2 is the same as stage 1 (including subclasses), but hearing loss, secondary to the tympanosclerosis, is present. In stage 3, the tympanosclerosis involves the middle ear only, but hearing loss due to tympanosclerosis is absent. Stage 4 is the same as stage 3, but hearing loss secondary to tympanosclerosis is present. This stage can be further categorized into subclasses related to the ossicles or joints involved (see Ossicular Fixation). In stage 5, the tympanosclerosis involves the tympanic membrane (with subclass designated) and middle ear, but no hearing loss is present. In stage 6, extensive tympanosclerosis involves both the tympanic membrane (stage 1-3) and the middle ear (with ossicle or joint involvement designated), and hearing loss secondary to tympanosclerosis is present.

Ossicular Fixation. As a sequela of chronic middle ear inflammation, the ossicles can become fixed, usually by fibrous tissue, which in turn is caused by adhesive otitis media, tympanosclerosis, or both, each of which has a staging system for extent and presence or absence of hearing loss. The ossicle itself or 1 or both of the joints (incudostapedial or incudomalleal) may be fixed. A classification of ossicular fixation is related to site of pathology, such as 1) stapes footplate, 2) incudostapedial joint, 3) incus, 4) incudomalleal joint, and 5) malleus.

Infectious Eczematoid Dermatitis. Perforation with otitis media and otorrhea (ie, acute otitis media with perforation and otorrhea or chronic suppurative otitis media) can cause an infection of the external auditory canal called infectious eczematoid dermatitis. An alternative term is infectious eczematoid external otitis. Classification is related to duration. In acute infectious eczematoid dermatitis, the duration of the external auditory canal infection is less than 3 months. In chronic infectious eczematoid dermatitis, the duration of the external auditory canal infection is 3 or more months.

INTRACRANIAL COMPLICATIONS OF OTITIS MEDIA

There are 7 intracranial suppurative complications of otitis media, any of which can caused not only by otitis media, but also by either intratemporal complications, such as mastoiditis and labyrinthitis, or 1 or more of the other complications of otitis media within the intracranial cavity.

Meningitis. Meningitis is an inflammation of the meninges that, when it is a suppurative complication of otitis media or certain related conditions (eg, labyrinthitis), is usually caused by a bacterium associated with infections of the middle ear, mastoid, or both.

Extradural Abscess. Extradural abscess is an infection that occurs between the dura of the brain and the cranial bone; it is also termed epidural abscess.

It usually results from the destruction of bone adjacent to dura by cholesteatoma, chronic suppurative otitis media, or both. This occurs when granulation tissue and purulent material collect between the lateral aspect of the dura and the adjacent temporal bone. Dural granulation tissue within a bony defect is much more common than an actual accumulation of pus. When an abscess is present, a dural sinus thrombosis or, less commonly, a subdural or brain abscess may also be present.

Subdural Empyema. A subdural empyema is a collection of purulent material within the potential space between the dura externally and the arachnoid membrane internally. Since the pus collects in a preformed space, it is correctly termed empyema rather than abscess. Subdural empyema may develop as a direct extension or, more rarely, by thrombophlebitis through venous channels.

Focal Otitic Encephalitis. Focal otitic encephalitis (the term cerebritis is synonymous) is a potential suppurative complication of acute otitis media, cholesteatoma. or chronic suppurative otitis media, or of 1 or more of the suppurative complications of these disorders, such as an extradural abscess or dural sinus thrombophlebitis, in which a focal area of the brain is edematous and inflamed.

Brain Abscess. Otogenic brain abscess is an intracranial suppurative complication of cholesteatoma or chronic suppurative otitis media, or both, but may also be caused by acute otitis media or acute mastoiditis. In addition, an intratemporal complication, such as labyrinthitis or apical petrositis, may be the focus, or a brain abscess may follow the development of an adjacent intracranial otogenic suppurative complication, such as lateral sinus thrombophlebitis or meningitis. Brain abscess can be classified related to 1) its site in the brain, such as the temporal lobe or cerebellum; 2) whether it has solitary or multiple lesions; and 3) whether the lesions are well defined or ill defined (cerebritis), which is related to management and outcome.

Dural Sinus Thrombosis. Lateral and sigmoid sinus thrombosis or thrombophlebitis arises from inflammation in the adjacent mastoid. The superior and petrosal dural sinuses are also intimately associated with the temporal bone, but they are rarely affected. This suppurative complication can occur as the result of acute otitis media, an intratemporal complication (eg, acute mastoiditis or apical petrositis), or another intracranial complication of otitis media.

Otitic Hydrocephalus. Otitic hydrocephalus is the term that describes a complication of otitis media in which there is increased intracranial pressure without abnormalities of cerebrospinal fluid. The pathogenesis of the syndrome is unknown, but since the ventricles are not dilated, the term benign intracranial hypertension also seems appropriate. The disease is usually associated with lateral sinus thrombosis.

CONCLUSIONS

As can be seen from this classification, which provides definitions, terminology, grading, and stages of otitis media and its complications and sequelae, the clinician-investigator must define the specific disease or disorder being managed or studied. Also, there is a critical need to establish universal consensus regarding the definitions, grading, and staging of many of these disease entities, which hopefully will initiate discussion and consensus-building in the future. The following are important issues in this endeavor.

1. When conducting and reporting studies of otitis media, the definition of the specific disease being investigated should be precisely defined.
2. Grading and staging of otitis media, its complications, and its sequelae is important not only for studies, but also for clinical management.
3. The definition of acute otitis media is distinctly different from the definition of otitis media with effusion. The distinction has clinical implications related to the need to treat and to the incidence of complications and sequelae of each condition.
4. The term otitis media with effusion is preferable to other terms, such as secretory, serous, or nonsuppurative otitis media.
5. The term middle ear effusion does not distinguish between effusions due to acute otitis media and effusions due to otitis media with effusion.
6. Despite the lack of a middle ear effusion, eustachian tube dysfunction is a disorder of the ear in which the signs and symptoms may be similar to those that are present when there is an effusion in the middle ear.
7. Perforations of the tympanic membrane should be classified according to their site, extent, and duration.
8. The term chronic otitis media is confusing, is potentially misleading, and should not be used, since some consider it to mean chronic otitis media with effusion, others think it means chronic suppurative otitis media, and still others include cholesteatoma under this term.
9. The terms "inactive" and "active" chronic suppurative otitis media are not recommended, since the disease can be a chronic perforation, with or without

acute or chronic otitis media. Chronic suppurative otitis media means a chronic perforation with chronic otitis media.

10. In the absence of signs and symptoms of mastoid infection, an effusion in the mastoid, as visualized on CT scans, does not necessarily indicate a need for mastoidectomy, since the effusion can be the natural extension of disease in the middle ear, such as an acute otitis media or otitis media with effusion.

11. The term subacute mastoiditis is preferable to the term masked mastoiditis. Regardless of the terminology, the condition should not be confused with an attack of acute otitis media that does not improve symptomatically despite antimicrobial therapy, ie, acute otitis media treatment failure.

12. Terms such as coalescent mastoiditis and acute surgical mastoiditis should not be used, since they do not appropriately describe the pathology — especially to non-otolaryngologists — of acute mastoiditis with osteitis.

13. The grading system proposed by House and Brackmann[14] is recommended when a facial paralysis occurs as a complication of otitis media.

14. A staging system of atelectasis of the middle ear is desirable related to the following factors: 1) duration (acute versus chronic), 2) whether it is localized or generalized, and 3) presence or absence of a retraction pocket.

15. A retraction pocket should be staged according to the following conditions: 1) whether it approximates or is adherent to middle ear structures, 2) whether it expands with positive or negative pressure, 3) whether its extent can be fully visualized, and 4) whether it is self-cleansing and free of infection.

16. The term cholesteatoma with chronic suppurative otitis media is appropriate when there is chronic infection in the middle ear cleft, but it is not appropriate when no otitis media is present.

17. Cholesteatoma should be staged according to the following conditions: 1) extent, 2) presence or absence of ossicular erosion (and number of ossicles), and 3) whether the disease is completely amenable to surgical removal.

18. Other sequelae, such as cholesterol granuloma, adhesive otitis media, and tympanosclerosis, should be staged.

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2. EPIDEMIOLOGY, NATURAL HISTORY, AND RISK FACTORS

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This report marks the 20th anniversary of the first Epidemiology and Natural History Panel Report. Epidemiology has become an increasingly important discipline in the study of otitis media (OM) over the past 2 decades. The objective of this report is to provide a critical review of the epidemiologic literature that has appeared since the 1995 Panel Report, and to identify short-term and long-term goals relevant to OM epidemiology, natural history, and risk factors.

RECENT SIGNIFICANT CONTRIBUTIONS

International Otitis Media Prevalence and Incidence. Prevalence rates of otitis media with effusion (OME) and chronic otitis media (COM) in preschool and school age children have recently been reported from developed and less-developed countries, including Greenland,[1] Thailand,[2] Malaysia,[3] Tanzania,[4] Greece,[5] Hong Kong,[6] Kenya,[7] Vietnam,[8] Jamaica,[9] and Italy.[10] These rates vary widely, but it is difficult to directly compare results across countries because of differences in design and population characteristics. Nevertheless, these studies generally confirm the much-reported finding that OME prevalence decreases with age. However, among Vietnamese children, the age-specific prevalence of a type B tympanogram decreased until age 7 years and then increased between ages 8 and 10.[8] The authors postulate that higher OME rates in the 8- to 10-year-olds were the result of an increased rate of upper respiratory tract infections from working in the rice fields. In Hong Kong, the rates of OME were 7 times higher in white 5- to 6-year-olds than in same-aged Chinese children,[6] and Inuit children in Greenland[1] and Canada[11] appear to have 5- to 10-fold higher rates of COM than other similar-age populations. Studies of Australian Aboriginal infants document that 95% have OM onset (symptomatic or asymptomatic) by 2 months of age, with the majority of cases progressing rapidly to COM.[12,13]

Trends in Otitis Media Incidence Over Time. Population-based studies of acute OM from Finland[14] and recurrent OM from the United States[15] reported marked increases in the incidence of these diseases (68% in Finland and 39% in the United States) over the past 10 to 20 years. Although the increased incidence of OM is consistent with increased attendance in group day care in both countries over this time period, these reported trends should be interpreted cautiously. Factors that could affect the diagnosis of OM, such as changes in health care systems, access to and use of care, and parent and physician awareness of OM and its risk factors may have occurred over the same period. Furthermore, criteria for diagnosis of OM may have changed, resulting in more cases of mild OM being diagnosed now than in earlier years.

An increased incidence of acute mastoiditis related to insufficient antibiotic treatment of OM has been reported.[16] Prolonged times between symptom onset and treatment were noted for the majority of patients with intratemporal and intracranial OM complications in an uncontrolled study.[17] Retrospective studies showed a shorter duration of hospitalization and less likelihood of OM history in acute mastoiditis patients seen recently than in those seen 10 to 20 years ago.[18,19]

Race, Social Class, Age, and Gender. Previous clinical research using passive surveillance and national survey data[20] has reported lower risks for acute OM, recurrent OM, and OME for African American children than for white children. However, several recent studies using frequent examinations and objective assessment criteria have reached different conclusions. Casselbrant et al[21] have shown similar rates of both acute OM and middle ear effusion (MEE) in African American and white children examined monthly and followed to age 2. African American children had a longer cumulative time with MEE during the first and second years of life than did white children in a study by Paradise et al,[22] but racial differences disappeared when study site (urban, suburban, rural) was controlled. In addition, data from the Third National Health and Nutrition Examination Survey (NHANES III), which used flat tympanograms to assess OME, found that white and African American school-age children had similar rates of OME, while Hispanic children had higher rates.[23]

Among children followed from birth to 2 years, a longer cumulative duration of OME was associated with lower socioeconomic status in a multivariate model controlling for race and other factors (based on mother's level of education and health insurance).[22] Similarly, in a cross-sectional study in Greece,[5] school-age children of less-educated mothers had higher rates of OME than same-age children whose mothers were more educated.

Both clinic- and population-based investigations published since 1995 have reiterated the findings of many previous studies: young age, age at onset, and male gender are significant risk factors for recurrent OM,[20,24-27] OME and cumulative time with OME,[5,22,28,29] and treatment with tympanostomy tubes.[30] However, Alho et al[31] reported that male gender, but not an early first episode, increased the risk of chronic OME.

Day Care, Exposure to Other Children, and Respiratory Infections. Most recent studies evaluating child care and OM report that exposure to other children at home or in a day-care setting increases the risk of acute OM, recurrent OM, and OME. Recent studies confirm that risk increases as the number of children in the setting increases, particularly among children under age 2.[22,24,28,32-34] Postma et al[30] showed a significantly increased risk of first and second tympanostomy tube insertions in children in day care as compared to control children. Kvaerner et al[35] found that the most important risk factors in early acute OM among Norwegian infants were attendance at day care with many children and having siblings in day care, and that recurrent OM was associated with having siblings and attending day care. Daly et al[33] reported that day care and siblings, but not siblings in day care, increased the risk of early OM onset. These dissimilar findings probably reflect the fact that many children in the United States enter day care in the first few months of life, whereas children in Norway typically enter day care at about 1 year of age. A single retrospective study reported that day care alone was not a risk factor, but that the combination of day care, allergy, and passive smoking increased the risk of recurrent OM.[36] An increased risk of OME for children with many siblings has also been reported.[22,28,29,34]

A US study using data from the 1988 Child Health Supplement reported that among children less than 6 years of age who were cared for outside the home, center care and the number of children in the setting were significantly associated with frequent episodes of OM, whereas the number of hours per week in child care was not.[37] Among 1- to 2-year-old children, frequent OM was significantly associated with variables measuring exposure to many children (≥4 in the child-care setting, changes in child-care arrangements, and use of center care as the first placement). However, no significant effects for these factors were observed among 3- to 5-year-olds. A longitudinal study of OME among African American children attending a day care center reported that the prevalence of bilateral OME declined from 12% at 2 to 2.5 years to 4% at 4.5 to 5 years.[38] These findings suggest that the risk of OME associated with day care diminishes as the child approaches school age.

Studies have demonstrated that center care also increases the risk of upper and lower tract respiratory infections and OM in the first 2 years of life.[29,34,39] Both day care and respiratory infection are independent risk factors for early OM onset,[33] probably because day care facilitates both colonization and active infection. Upper respiratory tract symptoms, infections, and OM are also associated with day-care attendance among 3- to 5-year-olds, but rates of respiratory infection and OM declined with age among Finnish 1- to 7-year-olds.[39] Older preschoolers who attend group day care appear to be less prone to respiratory infections and concomitant bouts of OM than infants and toddlers. Heikkinen and Ruuskanen[40] identified respiratory infection as the most important risk factor for OM and asserted that other OM risk factors are actually predisposing factors for respiratory infection. They advocated implementing strategies to decrease the spread of respiratory viruses by targeting many of the known risk factors for OM (day care, parental smoking, breast-feeding duration), as well as by using antiviral and antibacterial agents and vaccines.

Breast-Feeding and Passive Smoke Exposure. Studies of various designs continue to report dose-response relationships between duration of breast-feeding (or bottle-feeding), and/or exposure to smoking and OM[5,22,41,42] Nevertheless, there are also a large number of negative studies.[20,26,29,31,34,43,44] Two studies have reported that heavy, but not moderate or light, maternal smoking increases OM risk in early life.[42,45] Homoe et al[46] showed a significant *positive* association between duration of breast-feeding and later OME among Greenland Inuit children that may be related to socioeconomic factors. Some of these studies may have inadequate power to demonstrate significant relationships, and in others, interrelationships between smoking, breast-feeding, and social class may confound analysis. Furthermore, exposure to smoking, usually assessed by maternal report of parents' smoking status, may not adequately measure the child's actual exposure. Dose and duration issues may also be important in the relationship between smoking and OM.

In studies from Buffalo,[41,47] the rate of nasopharyngeal colonization at age 6 months, but not at 3 or 12 months, was significantly lower in exclusively breast-fed infants than in exclusively formula-fed infants, and colonization before 3 months of age was related to early OM onset. The same group reported that adherence of nontypeable *Haemophilus influenzae* to respiratory epithelium was not influenced by breast-feeding.[48] Swedish investigators[49] reported that activity of several types of breast milk antipneumococcal antibody against complex capsular polysaccharide, C-polysaccharide, and cell wall polysaccharide did not prevent nasopharyngeal carriage of pneumococcus or subsequent acute OM. Women whose breast milk had antibody activity against cell wall polysaccharide actually had significantly higher rates of carriage than those without antibody activity (28% versus 14%).

Genetic Susceptibility. Reported racial differences[23,46] and familial aggregation[26,33,50] suggest genetic susceptibility in OM. Twin studies from Norway[51] and Pittsburgh[52] have reported that OM has a large heritability component. The Norwegian study, applying multivariate modeling, found that genetic factors are major contributors to OM. Heritability was estimated at 74% for females and 45% for males, with common environmental effects accounting for another 29% of the variability for OM in males. A prospective study by Casselbrant et al[52] confirmed the substantial genetic variability of OM and reported a heritability of 72%, with no difference between males and females. A segregation analysis of first-degree relatives from 176 families suggests that OM susceptibility is likely to be due to more than 1 gene.[53]

The effect of age at first acute OM episode[1,20,25,43] and male gender[22,43] on subsequent otitis-proneness may be related to genetically determined anatomic or physiological variability. It has also been suggested that the high carriage rates of nonencapsulated *H influenzae* (NCHi) in Australian Aboriginal infants resulting from concurrent and successive colonization with multiple strains is related to immune suppression or tolerance in the host.[54] Evidence that some children are particularly susceptible to many types of upper respiratory tract infections also suggests a genetically determined defect in immunocompetence.[55] No new reports on the impact of variations in nasopharyngeal dimensions on OM susceptibility have been published to add to earlier reports.[56] Additional research is needed to understand the extent to which genetic and environmental influences are important in determining phenotypes.

Prenatal and Perinatal Factors. Earlier studies often have not demonstrated an association between low birth weight (<2,500 g) and OM risk, possibly because low birth weight is an uncommon outcome in the industrialized countries in which the studies were conducted. However, recent evidence suggests that very low birth weight (<1,500 g) or very preterm birth (<33 weeks of gestation) increases the risk of OM. Engel et al[57] followed normal-weight infants and high-risk infants in neonatal intensive care units for 2 years and found that the high-risk infants had a 20% higher prevalence of OME between 6 months and 2 years of age than did the normal-weight infants. Using longitudinal data from the National Maternal and Infant Health Survey, which oversampled very low–birth weight infants, Nalluswami et al[58] reported that these infants were at a 40% increased risk of frequent OM in the first 3 years of life, after adjusting for other OM risk factors. Consistent with these findings, intrauterine growth retardation was found to be significantly associated with recurrent OM in a large population-based sample of Norwegian twins.[59] The twin with the lower birth weight was more likely to have recurrent OM than the heavier twin. This study used perinatal data from the Norwegian Medical Birth Registry and responses about recurrent OM on a questionnaire submitted when the subjects were 18 to 25 years old. In a study designed to investigate prenatal, infant, and environmental risk factors for OM onset in the first 6 months of life, infants with cord blood antibody levels to *Streptococcus pneumoniae* types 14 and 19F in the lowest quartile had significantly earlier onset of OM than infants with higher antibody levels.[60] However, maternal nutrition, symptoms and illnesses, and use of drugs, alcohol, medications, and dietary supplements during the last trimester were not related to early OM onset.[33] In a retrospective study comparing otitis-prone cases with controls, there were no differences in pregnancy or delivery complications, prematurity, or birth weight.[26]

Other Risk and Protective Factors. Studies from Finland have shown that pacifier use is a risk factor for OM.[61] In a randomized intervention study comparing 2 clinical populations, lower rates of acute OM were reported in the intervention group, in which parents received advice to reduce pacifier use, than in the control group.[62] Brook and Gober[63] cultured pacifiers of children with acute OM and concluded that because pacifiers did not carry a large number of organisms, they were unlikely to play an important role in transfer of pathogens. In another randomized trial of children in day care, use of xylitol gum or syrup decreased the risk of acute OM in the intervention group,[64] but did not provide resolution of MEE.[65] An association with prone sleep position was first reported at the 1995 Recent Advances in Otitis Media meeting. This finding was replicated in 2 large studies in the United Kingdom, as reported at the 1999 meeting.[66] A study examining vitamin intake and OM did not find a relationship between baseline serum retinol and carotenoid levels and OM incidence in 3- to 5-year-olds.[67]

Among multiple indoor environmental factors investigated in a study of school-age children in China, only living in damp homes was associated with acute OM.[44] No associations were found for other risk factors, including number of siblings, bottle-feeding, and parental smoking. Another study found that the proportion of primary-school children with OME was significantly related to distance from industrial emissions stacks (≤1,000 m versus >1,000 m) when socioeconomic status was controlled for, but this relationship did not persist when distance was examined as a continuous variable.[68] Parental smoking and other risk factors were not investigated. Using interview data from the Third National Health and Nutrition Examination Survey, MacTurk et al[20] found that a ponderal index at or below the 10th percentile was significantly associated with reported frequent OM in children less than 6 years old; the study controlled for gender, race, income, maternal smoking, breastfeeding, day care, and child health status.

Natural History, Complications, and Sequelae. According to Alho et al,[31] acute OM episodes constituted the greatest risk for chronic OME. The risk was transient and highest just after the acute episode, but eventually disappeared after 3 months. After 2 successive episodes, the risk for chronic OME was 30-fold higher, and it was almost 40-fold higher after 3 successive episodes. Stenstrom and Ingvarsson[69] examined a cohort of otitis-prone children at 3 to 7 and at 9 to 14 years of age. Although the rates of OM assessed with tympanometry decreased over this period, the rates of tympanosclerosis and COM increased.[68] Daly et al[70] conducted a cross-sectional study of sequelae among children, adolescents, and adults at 4 years and at 9 to 23 years after tympanostomy tube treatment. The older cohort was significantly more likely to have severe retractions, hearing loss, and atrophy affecting more than 50% of the eardrum, but were less likely to have flat tympanograms than the younger cohort. Schilder et al[71] reported on a group of 63 tube-treated 7- to 8-year-old children who were matched on OM history, gender, and age with a group of children who were not treated surgically. Tympanosclerosis and atrophy were significantly more common in the tube-treated children, but hearing, language, reading, and spelling measures were similar in the two groups.[71] Strachan et al[72] compared age-matched children treated with either Shepard tubes or T-tubes. The groups were comparable with respect to indications for surgery and type of fluid, and at 6 to 10 postoperative years, hearing, rates of retraction, and rates of tympanosclerosis were similar, but rates of perforation were significantly higher in the T-tube group (24% versus 2%).[72] Stangerup et al[73] compared the prevalence of tympanic membrane pathology in 2 Danish cohorts born in 1955 and 1975. The older cohort, examined at age 35, had a significantly lower incidence of pars tensa pathology than did the younger cohort (6% versus 17%). The older cohort was never treated with grommets, whereas the younger cohort had a high probability of grommet insertion. In both cohorts, the risk of eardrum pathology was related to a history of acute OM and/or OME.

Methodological and Analytical Strategies. Since the 1995 meeting, a major advance has been the increased use of multivariate analysis strategies to control for confounding factors and estimate relative risks and odds ratios in epidemiological studies. These strategies include multiple logistic regression (most common), dynamic logistic regression for time-dependent variables, multiple linear regression, Cox regression, and Poisson regression. In addition, although meta-analysis has been used for several years to evaluate randomized trials for OM treatment, this analytic technique has just begun to be applied to the evaluation of risk factors for acute OM, recurrent OM, and OME. In performing meta-analysis, it is important to use high-quality studies with standard criteria and definitions, and to determine ways to deal with publication bias and lack of homogeneity in study designs. Finnish researchers completed a meta-analysis of OM risk factor studies,[74] and Rovers et al[75] did a critical overview of day care as a risk factor. Both groups confirmed the importance of day care. In the study of Rovers et al, comparing center care to home care produced the largest effect, with less difference in risk between family care and home care. In the Finnish study, in addition to day care (RR = 2.5), other significant risk factors in the meta-analysis included family OM history (RR = 2.6), parental smoking (RR = 1.7), breast-feeding for 3 or 6 months (RR = 0.87), and pacifier use (RR = 1.2, based on only 2 studies). DiFranza and Lew[76] conducted a meta-analysis of 28 studies of children's exposure to smoking and middle ear disease (measured by acute OM history, diagnosis of acute OM or OME, or abnormal tympanometry or audiometry) to compute pooled odds or risk ratios and a population-attributable risk using population-based data on children's exposure to passive smoking. Included studies used parental smoking, number of household smokers, number of cigarettes smoked in the home, and cotinine levels as measures of the child's exposure. The estimated pooled relative risk was 1.19 (confidence interval [CI], 1.05-1.35), and the pooled odds ratio was 1.58 (CI, 1.11-2.24), with a population-attributable risk of 2% to 13% or 354,000 to 2.2 million cases of middle ear disease each year attributable to exposure to cigarette smoke in the United States. Alho et al[77] also calculated attributable risks for children from birth to 2 years in Finland, using their population-based sample. They concluded that OM incidence would be reduced by 19% by eliminating center care, with a 42% reduction in recurrent OM. Breast-feeding for more than 3 months could decrease OM by 3% and recurrent OM by 6%, and eliminating smoking would result in a 3% decrease in OM and a 22% decrease in recurrent OM. Louhiala et al[39] estimated that 50% of OM among 1-year-old Finnish children was attributable to center care.

Short-Term Goals

1. Otitis media susceptibility has been shown to have a large heritability component. Genes associated with OM in families should be identified with linkage and candidate gene studies.

2. To explore anatomic, physiological, and immunologic theories supporting the hypothesis of genetic susceptibility, we need data to determine the relative importance of genetic and environmental factors.

3. Recent studies suggest that OM incidence has increased over the past 10 to 20 years. The following research would clarify these findings: repeating population-based studies done in Denmark in the 1970s by using the same methodology to determine changes over time in OME prevalence; investigating reasons for the apparent increase in OM incidence, including societal, parental, and physician factors; exploring the postulated increase in the incidence of acute mastoiditis; and confirming the reported relationship between time from symptom onset to treatment and mastoiditis and other complications.

4. Use of multivariate analysis in risk factor studies has increased over the past 4 years, but should be used universally in future studies to control for confounding factors. Control of social class seems to be particularly important in studies examining race, breast-feeding, and smoking.

5. To better understand the natural history of acute OM and OME, more studies of long-term sequelae — particularly middle ear and tympanic membrane pathology — are needed. Video-otoscopy is well suited to tracking changes in ears over time.

6. The majority of studies investigating risk factors have focused on the first 2 years of life. More studies are needed to characterize risk factors and determine the magnitude of their effect as the child ages. For example, recent reports show that the relationship between OME, frequent OM, and day care diminishes as the child reaches 3 years of age.

7. Although encouraging reports about OM prevention strategies have been reported since the last Recent Advances meeting (ie, xylitol gum and reduced pacifier use), more research on prevention strategies, particularly among young children in day-care settings, is needed. This is particularly important given the troubling rise in antibiotic-resistant organisms over the past 5 to 10 years.

8. Definitions of recurrent OM are widely divergent, making it extremely difficult to compare results across studies. We request the Committee on Definitions and Classifications to propose a standard definition for recurrent OM to be used by researchers to enhance study comparability across populations.

9. Prevalence of eardrum pathology and OM histories should be determined in cohorts born before and after tubes were commonly used to treat OM to examine the individual and joint effects of OM and treatment with tubes, and look for evidence of changing OM incidence over time.

Long-Term Goals

1. Risk factors for OM need to be carefully characterized and mapped so that a child at high risk for recurrent and persistent OM can be identified early. Identification could result in primary and/or secondary prevention of OM, decreasing complications and sequelae.

2. Ultimately, discovery of a gene or genes linked to OM susceptibility would allow characterization of gene mutations, loss of function alleles, and gene products. These discoveries could provide new insights into the pathogenesis and mechanisms of the disease etiology.

3. Future population-based studies should, when possible, use objective tests (eg, tympanometry, pneumatic otoscopy) rather than parental reports to assess the prevalence of OM and OME.

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3. MIDDLE EAR PHYSIOLOGY AND PATHOPHYSIOLOGY

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PANEL OBJECTIVES

1. Describe important new contributions to our understanding of middle ear (ME) physiology and pathophysiology as they relate to otitis media (OM) and, in specific, to the research goals identified at the previous meeting of this Panel.[1]

2. Identify deficiencies in our understanding of ME physiology and pathophysiology.

3. Define priority research goals that address those deficiencies for study within the next 4 years.

4. Identify the long-range goals of this research focus and suggest ways in which they can be implemented.

In this discussion, we present a summary of selected studies conducted within the past 4 years that we believe have contributed to our understanding of ME physiology and pathophysiology. Here, we consider the ME system to include the tympanum, mastoid, eustachian tube (ET), and paratubal musculature. The reviewed material is not intended to be wholly inclusive, but to supply sufficient references so that interested clinicians and researchers can familiarize themselves with the various topics presented. Also, citations to published works do not necessarily reflect endorsement of the study's results and conclusions by all members of this Panel. For each topic, the goals identified in the previous report of this Panel[1] that were specifically addressed in the past 4 years are listed.

FORM-FUNCTION RELATIONSHIPS

(See Doyle et al, Short-Term Goals 3, 4, 6, and 11, pp 16-17.[1])

The form-function paradigm continues to be used to explore the constraints imposed on ET function by the anatomy of the tube and related structures. There, osteological studies of cranial anatomy reported new evidence showing a relationship between certain cranial base dimensions and indicators of past OM.[2] Other studies provided new descriptions of the musculature surrounding the ET[3,4] with particular reference to its role in ET function for normal and cleft palate populations. Also, an analysis of mucosal morphology suggested the existence of discrete regions of the ME with different resistances to diffusive gas exchange.[5]

By means of standard histopathologic techniques, a number of developmental changes in the ET were described and related to function. For example, the path length along the ET lumen was reported to grow from 21.2 mm in 3-month-old infants to 37.0 mm (average) in adults. A developmental shift in the orientation of the cartilaginous portion with respect to the bony portion was noted. In children, the cartilaginous and bony portions are both aligned to a line connecting the pharyngeal and the tympanic orifices, while in adults, the cartilaginous portion is angled inferiorly and laterally from the bony portion, resulting in a curved trajectory.[6] Also, there is a marked age-associated difference in the shape of the lumen of the cartilaginous portion. In adults, the cross-sectional area of the lumen decreases monotonically from the pharyngeal orifice to the narrowest portion, near the posterior end of the cartilaginous ET. In children, the lumen is uniformly narrow over approximately 80% of its length.This immature morphology may confer an increased risk of development of OM during childhood.[7] Finally, the depth of the epithelial folds in the ET decreases significantly as a function of age.[8] Since folding provides a relatively greater epithelial surface area, it could promote greater ME clearance by action of ciliated cells in the folded regions of the epithelium. Folding might also support the protective function of the ET by maintaining a narrow lumen.

New findings have contributed to our understanding of the potential roles of the levator veli palatini muscle (LVPM) in opening the ET. Five key features have been identified in the anterior cartilaginous portion of the tube, ie, the LVPM is always located inferolateral to the inferior margin of the medial lamina of the tubal cartilage; the LVPM has a large cross-sectional area; the medial lamina and the LVPM are in close proximity; the tensor veli palatini muscle (TVPM) is not attached to the lateral lamina over the anterior quarter of the cartilaginous ET[9]; and posteriorly, the LVPM is located inferior to the medial lamina. These observations suggest that the most anterior portion of the ET is opened by contraction of the LVPM, which rotates the superomedial portion of the medial lamina.[10]

Other studies showed that normal infants have a significantly smaller ET cartilage than adults and that infants with cleft palate have a poorly developed lateral lamina.[11] Rosenmuller's fossa, with rich lymphoid tissues, was found to extend along the tube as far as one half of the total length of the ET, and very close to the skull base near the foramen lacerum.[10] These findings suggest that infection in Rosenmuller's fossa can invade the cranial cavity via the foramen lacerum.

A number of in vivo methods for the study of ME anatomy have been explored. By means of a superfine fiberoptic endoscope introduced through the ET, the ME of cadaveric specimens was visualized, and the potential feasibility of this method for in vivo use in humans was thereby documented.[12] Also, with an endoscope, the nasopharyngeal orifice of the ET was studied in children and adults with OM. In both children and adults, blockage of the ET was commonly associated with mucopurulent nasal discharge, although other causes were also reported.[13] In a related study, the cause of tubal constriction with swallowing was found in adults to be most often associated with inflammation of the pharyngeal orifice of the ET, and in children to be associated with a squeezing of the tubal orifice during palatal elevation.[14] The value of computed tomography (CT) and magnetic resonance imaging (MRI) has been established for study of 1) the anatomy of the ME,[15-17] 2) the geometric structure of the mastoid air cell system,[18] 3) the anatomy of the ET and paratubal musculature in normal and pathological conditions,[19-21] 4) the diagnosis of OM,[22-23] and 5) the pathological changes in the ME associated with various diseases.[24-26] Magnetic resonance imaging has also been used as a gold standard for assigning sensitivity and specificity to other methods of diagnosing ME effusion, such as tympanometry, otoscopy, and audiometry.[27-29] Continued developments in these areas promise a more complete description of the in vivo development of ME disease in response to various preconditions, more accurate diagnosis of OM, and a better understanding of the relationship between ET structure and ET function.

SHORT-TERM GOALS

1. Continue with studies of the growth and development of the ET system by both standard histologic methods and the newer radiologic methods.

2. Correlate the results of currently available tests of ET function and those of concurrent MRI evaluations of ET morphology and evaluate hypotheses regarding mechanisms of tubal dilation.

3. Clarify whether the hinge region between the medial and lateral laminae of the ET cartilage is more responsible for ET opening than is the the lateral movement of the membranous wall.

4. Relate in vivo ET form-function analysis for an individual to the subject's predisposition to OM.

ET PRESSURE-REGULATING AND CLEARANCE FUNCTIONS

(See Doyle et al, Short-Term Goals 1, 3, 4, 5, 6, 7, 8, and 13 and Long-Term Goal 2, pp 16-17.[1])

The pressure-regulating (ventilatory) function of the ET continues to be a main focus of research. Many studies assessed tubal function from a single measurement, but relatively few focused on the short- and long-term variabilities of tubal function on the individual level. Recently, tubal function was assessed by forced opening, pressure equalization, Valsalva, and sniff tests in children treated with tympanostomy tubes after secretory otitis media.[30] The intraindividual within- and between-test (30 minutes) variabilities in the forced opening and closing pressures were small. The longitudinal data showed the opening and closing pressures to increase over the 4 months from the day of tube insertion, but then to remain stable. In contrast, the results of active tubal function tests showed a different pattern, ie, a large test-retest variability, but no patterned change, after tube insertion.

New techniques have been developed that have the potential to noninvasively assess tubal patency and function; they include the visualization of a radioactive gas introduced into the ME during procedures that force ET opening.[31,32] Also, a method for the direct measurement of tubal compliance was described and was used to discriminate normal from diseased MEs.[33] The compliance of the tube was shown to be affected by mucosal surface tension in an experimental study suggesting that surfactants facilitate tubal opening.[34] Other studies measured the pressure-regulating function of the tube in different populations at risk for OM[35-38] and after radiotherapy of nasopharyngeal carcinoma.[39,40] Assessment of that function was shown to predict a subject's ME response to hyperbaric oxygen treatments[41] and was used to screen subjects for tympanostomy tube insertion.[42] Also, 2 studies in monkeys showed that certain test parameters reflecting ET pressure regulation were responsive to changes in both the local and systemic partial pressures of oxygen and carbon dioxide, probably because of changes in the degree of mucosal venous congestion.[43,44]

Gas transfer across the ET has been modeled in a number of reports.[45,46] The required parameters for those models are the gas composition of the nasopharynx (source gas for ME influx), the relative frequency of ET opening, and the effective ratio of the volume of gas introduced during tubal openings to the volume of gas lost between openings. Two recent experimental studies addressed the first issue and provided convincing evidence that the gas introduced into the ME during tubal openings is of approximate end-expiratory composition.[47,48] Thus, bolus gas transfer from the nasopharynx to the ME is not expected to alter significantly the ME gas composition, as the compositions of both the source and the sink are very similar. A unique experimental format consisting of continuous, long-period (24-hour) measurement of ME pressure was used to estimate the frequency of pressure changes identifiable as ET openings. The data were interpreted as showing that tubal opening was rather infrequent, despite the maintenance of near-ambient ME pressure.[49,50] However, those results conflict with the data for an alternative sonotubometric method of continuously monitoring ET openings that reported opening frequencies of approximately 1 per minute in healthy subjects.[51] Also, a clinical study reported differences in the gas volume input-output ratio between a group of ears that maintained normal pressures and a group that developed underpressures over the course of an experiment. Specifically, no differences were observed between groups in the volume of gas loss, but significant differences were observed in the frequency of tubal openings and in the total volume of gas gain.[52] Taken together, these results suggest that for healthy MEs, the demand placed on the ET for resupply of gases is much less than previously believed, and that the normal ME will maintain near-ambient pressures with ET openings as infrequent as 1 per day.[48]

The ET clearance function remains understudied, with most investigations focusing on evaluating experimental therapies in animal or in vitro models (see below). Two studies have addressed relatively new issues regarding the clearance function. The first used guinea pigs and documented an impaired ME clearance via the ET during both local and systemic allergic reactions. These results may help explain the contribution of allergy to OM pathogenesis.[53] The second provided evidence supporting a dependence of the clearance function on the active opening function. There, in monkeys, clearance of fluid through the ET was shown to be ineffectual when the TVPM function was compromised.[54]

A number of studies have furthered our understanding of the effects of viral upper respiratory tract infection (URI) on ET function and ME pressure. In children followed longitudinally, the values of the tubal compliance index increased during periods of a URI, suggesting that the luminal mucosa is swollen during those infections.[55] Daily tympanometry in children during the winter months showed that URIs were temporally associated with the development of ME underpressures and OM with effusion,[56,57] but that the underpressures and effusions could precede by days the symptomatic presentation of a cold — an observation supported by cross-sectional epidemiological studies.[58] Similarly, for experimental influenza or rhinovirus infections in adults, the development of signs and symptoms of illness was not prerequisite to the development of otologic complications such as poor tubal function, ME underpressures, and earache.[59] These observations are important, in that they suggest there may not exist an obvious signal that defines a window for prophylactic intervention to prevent OM during a viral URI. Also, in experimental influenza in adults, the preexistence of a constitutionally good ET function was shown to be protective for the development of influenza-provoked ME underpressures,[60] and, like rhinovirus and influenza infection, experimental respiratory syncytial virus infection of adults provoked ET dysfunction and ME underpressure.[61] In a ferret model, experimental bilateral (but not unilateral) nasal obstruction caused the progressive, stepwise development of positive ME pressures during repeated swallowing. This could represent a mechanism for the introduction of nasopharyngeal pathogens into the ME during those periods of bilateral nasal congestion that accompany URI.[62]

SHORT-TERM GOALS

1. Determine the variability of the passive and active tubal functions as assessed by short- and long-term evaluations by different methods.

2. Compare the degree of variability in ears with different types of ME pathology and with healthy ears.

3. Determine to what degree Valsalva and sniffing maneuvers can be facilitated by muscular action when performed intentionally to achieve a desired sound quality or tympanic membrane position.

4. Define methods for noninvasively measuring ET clearance function and determine whether deficiency in that function is a sole and sufficient cause of ME effusion and/or a contributing factor to the persistence of ME inflammation.

5. Determine whether active muscle function is required for effective clearance of fluid via the ET, and whether that mechanism is consistent with the pumping function described previously.

6. Determine whether the ET response to viral URI and allergy is protective for the ME or whether it predisposes to OM by hydrops ex vacuo.

ME GAS EXCHANGE, MATHEMATICAL MODELS, AND PARAMETER ESTIMATION

(See Doyle et al, Short-Term Goals 1, 2, 10, 11, and 12 and Long-Term Goals 1 and 2, pp 16-17.[1])

In the past 4 years, the previously published, simple, 2-compartment mathematical models of transmucosal gas exchange have been refined to include representations of the diseased ME, ie, addition of mucosal and fluid compartments and increased mucosal blood flow.[45,56,63] Those revised models make specific predictions with respect to the behavior of ME pressure following air inflation in diseased versus healthy MEs. In experiments using monkeys and humans, those predictions were shown to be an accurate representation of system behavior.[22,64] There, the rapid decrease in pressure after air inflation of an ME containing effusion is caused by the redistribution of gas (primarily nitrogen) between the air space and the effusion. Also, methods for more accurate estimation of trans–tympanic membrane and transmucosal gas exchange parameters were developed.[46] The practicality of these methods was demonstrated in monkeys and guinea pigs.[65-67] For the tympanic membrane of monkeys, gas transfers were consistent with predicted rates of exchange across essentially a water barrier, and the volume of gas exchange across that structure was 1 (inert gas) or 2 (reactive gas) orders of magnitude less than the volume exchanged across the healthy ME mucosa.[65] For transmucosal exchange, the measured constants confirm the extremely slow rate of exchange of nitrogen (vis-à-vis that of the reactive gases) and predict a relatively inconsequential contribution of metabolism to ME gas partial pressures.[66] These results have implications for the expected gas composition of the ME, the rate of pressure change between ET openings, and the frequency of ET openings that is required to maintain near-ambient pressures. In experiments, the expectations based on the measured exchange constants were shown to be valid. Specifically, ME partial pressures of oxygen and carbon dioxide quickly respond to changes in their respective blood gas partial pressures[68,69]; the partial pressures of those gases for the normal and atelectatic MEs of humans are similar and are approximately equal to their respective mixed venous partial pressures[70]; and the rate of ME pressure decrease following ET obstruction is slow and on the order of 100 mm H_2O per day.[26]

The role of the mastoid in ME pressure regulation was explored in a number of experimental and clinical studies.[71-77] In pigs, an inhibited development of the pneumatized mastoid was caused by experimental inflammation of the ME mucosa, but not by ablation of the ME air space, and in humans with small mastoids, treatment of OM with tympanostomy tubes promoted a significant expansion of the mastoid air cell system.[71,72] These results support the concept that mastoid development is a consequence of and not a causal factor for the mucosal inflammation associated with OM. In contrast, others reported that in adults with mature mastoid systems, a small mastoid remained a risk factor for ME disease, although the interpretation of this observation is clouded by the omission of concurrent measurements of other known risk factors, such as poor ET function.[73,74] Three studies examined the relationship between mastoid size and the rate of ME pressure change in response to established ME-blood pressure gradients. The results showed that the rate of pressure change was directly related to the size of the pneumatized mastoid and that, after surgical reconstruction, mastoid gas exchange was dependent on the degree of mucosal preservation.[75-77] Those data were interpreted as evidencing a pressure-regulating function for the mastoid, associated with active gas influx into the ME. However, by means of the revised mathematical model of passive transmucosal gas exchange, that conclusion was shown to be a misinterpretation of the experimental conditions.[78] Specifically, the presented evidence for gas production by the mastoid was shown to be caused by the passive exchange of gas across the mucosa of a cavity characterized by a high surface area–volume ratio (mastoid) in response to experimentally induced partial pressure gradients. That model also defines the specific conditions under which the mastoid cavity would serve as a gas reserve. There, a gas reserve function is expected if the relative ratio of blood flow to surface area for the tympanum is very much greater than that for the mastoid cavity, with the absolute magnitude of the difference being directly related to the surface area–volume ratio of the mastoid cavity and tympanum.[79]

SHORT-TERM GOALS

1. Refine the existing models of transmucosal gas

exchange to describe the relevant transfers as noncompartmental, distributed processes.

2. Experimentally determine the transmucosal gas exchange constants for the normal and diseased MEs of humans.

3. Determine the significance of mastoid geometry to gas exchange in the air space and to the possible development of partial pressure gradients within the ME air space.

4. Measure in experiments the model parameter that determines whether the mastoid cavity is an air reserve.

5. Develop animal models to test the 2 alternative hypotheses relating mastoid size and OM.

6. Obtain better estimates of the frequency of tubal opening and the accompanying volume of gas transfer for healthy, otitis-prone, and diseased MEs.

MUCOSAL PHYSIOLOGY

(See Doyle et al, Short-Term Goal 8 and Long-Term Goal 1, pp 16-17.[1])

An important observation made previously was the initiation of ME mucosal changes at relative underpressures of approximately −200 mm H_2O, with effusion accumulation at slightly lower pressures. Recently, this finding was confirmed in both monkeys[26] and chinchillas.[80] To explore the cause of that effect, investigators exposed chinchilla MEs to varying gas compositions and pressures while mucosal blood flow and fluid leakage were measured concurrently. Neither measure was affected by gas composition, but both were affected by pressure.[80,81] While difficult to study in animals, the changes in certain aspects of mucosal physiology effected by a variety of stimuli, including relative hypoxia, free oxygen radicals, and other stressors, have been investigated in cell culture. For example, recent work using a cell line derived from gerbil ME mucosa focused on the potential role of ion transport in the pathogenesis of OM and the effects of various modulating agents on that process. The results of those studies showed that dexamethasone increases sodium ion transport[82] and increases the expression of α-subunit sodium channel messenger RNA.[83] These changes may help explain the efficacy of steroid treatment for OM. Other studies using variations of that model system demonstrated that 1) chronic hypoxia decreases sodium absorption[84]; 2) nitrous oxide potentiates the deleterious effects of oxygen[85]; 3) adenosine triphosphate increases intracellular calcium ions in a dose-dependent manner that correlates with an up-regulation of chloride ion secretion[86]; 4) *sai-rei-to*, a Japanese herbal remedy for OM, affects the selective activation of apical chloride ion channels[87]; and 5) extracellular adenosine activates the cAMP–protein kinase A system by an alternate mechanism that may involve the cystic fibrosis transmembrane receptor chloride ion channel.[88] Because these changes in mucosal physiology represent acute responses to stimuli, they may in part mediate and moderate the hydrops ex vacuo–provoked development of ME effusion by changing the fluid balance across the cell membrane and/or changing the persistence of effusion by changing the properties of the mucociliary transport system. Recently, a model system using primary cultures of rat ME epithelial cells was developed specifically to investigate the effects of altered pressures and gas compositions on cellular physiology, especially ion and fluid transport.[89]

SHORT-TERM GOALS

1. Determine the response of ME mucosal cell cultures to underpressures and changes in gas composition.

2. Evaluate this mechanism with respect to the transduction of deficient ME pressure regulation into OM.

PREVENTION AND TREATMENT OF OTITIS MEDIA WITH EFFUSION BASED ON KNOWLEDGE OF ME PRESSURE REGULATION

(See Doyle et al, Short-Term Goals 6, 9, and 13 and Long-Term Goals 3 and 4, pp 16-17.[1])

The prevention and treatment of ME disease remains an elusive goal, with most current medical therapies providing relatively marginal efficacies. In recent years, a variety of alternative approaches based on our current understanding of ME pressure regulation have been explored and have included the use of anti-inflammatory or antiviral treatments during viral URIs to prevent the consequent otologic complications; bioactive agents that promote improved ET opening and/or clearance function; and different methods to introduce gas into the ME and bypass the dysfunctional ET.

Regarding the medical treatments, rimantadine hydrochloride did not — but a neuraminidase inhibitor did — prevent the development of tubal dysfunction, abnormal ME pressures, and signs and symptoms of OM during experimental influenza A virus infection in adults.[90,91] Topical decongestants did not promote resolution of ME underpressures in children with viral colds or prevent barotitis in adults and children during air travel,[92,93] and topical intranasal surfactants failed to improve ME status in adults with colds. Studies using animal models and secondary outcomes (mucociliary clearance rate, tubal opening pressure, etc) have reported positive results for local topical surfactant treatment,[94] systemic anti-inflammatory treatment,[95] and treatments with different mucolytic agents and modulators of ciliary beat.[96,97]

Surgical implantation of a permanent mastoid cannula was described[98] as providing a good clinical response and as affording a possible alternative to tympanostomy tube insertion. Also, ME inflation has been revived as a treatment option both to prevent the development of significant ME underpressures and to treat the consequences of those abnormal pressures, ie, OM.[99] Recent studies in monkeys with experimental functional ET obstruction showed that the expected efficacy of ME inflation depends on the timing of treatment onset, on treatment compliance, and on treatment frequency.[100,101] Related studies showed that a diagnosis of disease resolution after inflation treatments can be an artifact of effusion displacement to the mastoid[23] and is dependent on the method of assessment. Theoretical modeling has shown the need for frequent early inflation and less frequent inflation later in the treatment course,[63] and this protocol was evaluated to be successful with respect to primary outcomes in monkeys and in a human with OM.[22,64] Unfortunately, clinical trials using the technique have reported contradictory results,[102-104] which may be related to differences among studies in treatment goals (prevention or resolution of disease), target populations (adults or children, etc), study designs, or treatment protocols.

To address these issues, to promote high-quality research into the efficacy of ME inflation, and to allow comparability and consistency across studies, the members of this Panel have established an ad hoc research committee on ME inflation for treatment of OM that has as its overall objective the scientific evaluation of air inflation for prevention and treatment of ME disease. Persons who have expressed an interest in participating are listed below, and any and all interested persons are invited to join.

The members of the Ad Hoc Committee on Middle Ear Inflation are Cuneyt M. Alper, MD; Charles D. Bluestone, MD; David J. Beste, MD; John P. Birchall, MD, FRCS; George G. Browning, MD, FRCS; Craig Buchman, MD; Marie Bunne, MD, PhD; Kenny Chan, MD; William J. Doyle, PhD; Debra M. Don, MD; Jens U. Felding, MD; Leif Hergils, MD; Mark P. Haggard, MA, PhD; Michal Luntz, MD; Bruce Maddern, MD; Paola Marchisio, MD; A. Richard Maw, MS, FRCS; Otavio Piltcher, MD; Sven-Eric Stangerup, MD; J. Douglas Swarts, PhD; Haruo Takahashi, MD; Mirko Tos, MD, PhD; and Birgit Winther, MD.

At the preliminary organizational meeting, participants recognized the need to develop single-center and multicenter protocols that explicitly address the following points.

1. Clear statement of treatment goal (prevention, resolution).
2. Delineation of target populations applicable to point 1.
3. Acceptance of theory-based treatment regimen (method of inflation, frequency, etc).
4. Definition of patient inclusion and exclusion criteria.
5. Description of clinically important outcomes (eg, restoration of hearing, resolution of pathology, etc).
6. Appropriate consideration of treatment compliance.
7. Rigorous analysis of the statistical power afforded by sample size, expected efficacy, etc.
8. Dissemination of both positive and negative results to a broad cross section of physicians.

Members of the Committee will discuss the various goals of the treatments, share experiences with respect to treatment methods and compliance, provide mutual assistance with respect to the design and interpretation of studies that bear on the stated objective, and exchange the results of their work in open and closed forums. While it is anticipated that the near-term studies will be primarily single-center, with all rights of priority and confidentiality given the project's investigator, future multicenter collaborative studies are foreseen with interested members participating.

SHORT-TERM GOALS

1. Continue to investigate promising interventions designed to prevent the development of OM during a viral URI.
2. Evaluate those agents shown to moderate ciliary beat frequency, ET clearance function, or ET pressure-regulating function by means of standard models of OM pathogenesis.
3. Conduct definitive, targeted clinical studies on the use of ME inflation for the prevention and treatment of OM.

LONG-TERM GOALS

The long-term objective of this research remains to better understand the physiology and pathophysiology of the ME system in relation to the pathogenesis of OM. To accomplish that objective, it is important that we refine our current models of ME pressure regulation, include as extensions to those models the mucosal changes that are precipitated at threshold underpressures, define rational interventions that reestablish adequate pressure regulation, and evaluate those interventions in the clinical population. The long-term goal of the research is to implement this paradigm and thereby define rational treatments for OM.

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4. ANATOMY, CELL BIOLOGY, PATHOLOGY, AND ANIMAL MODELS

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OBJECTIVE

The objective of this Panel was to identify important progress that has been made in the areas of cell biology, anatomy, pathology, and animal models in the past 4 years since the Sixth International Symposium on Recent Advances in Otitis Media, which was held in June of 1995.

GROSS ANATOMY AND TEMPORAL BONE HISTOLOGY AND HISTOPATHOLOGY

Sudo et al[1] studied normal temporal bones from persons 16 to 88 years old by computer-aided 3-dimensional (3-D) reconstruction and measurement. These results suggested that contraction of the tensor veli palatini muscle (TVPM) opens the narrowest portion of the eustachian tube (ET) lumen to ventilate the middle ear (ME) and that this portion also plays a role in protecting the ME.

In another study, Sudo et al[2] analyzed histologic sections from several temporal bones with ETs attached. They used a computer-aided 3-D reconstruction method in order to determine the curvature and tilt of the ET and the anatomic relationships among the medial lamina of the ET cartilage, the levator veli palatini muscle (LVPM), and Rosenmuller's fossa. They found that the ET is convex superomedially and is tilted medially. The LVPM is located inferolateral to the inferior margin of the medial lamina of the anterior ET cartilage. The LVPM is inferior to the medial lamina of the posterior ET cartilage. These findings suggest that the LVPM opens the anterior cartilaginous portion of the ET by rotating the medial lamina with increased dimension of the muscle body when it contracts.

Suzuki et al[3] investigated the postnatal development of the ET and its surrounding structures by means of computer-aided 3-D reconstruction methods in normal temporal bones obtained from individuals 3 months to 71 years old. They found that whereas in adults, the cross-sectional area of the lumen declined monotonically between a large opening at the pharyngeal orifice and the narrowest portion of the ET (near the border of the cartilaginous and junctional regions), in children, the ET lumen was uniformly smaller over the first 80% of its length from the pharyngeal orifice. The investigators suggested that this immature morphology of the ET lumen may confer an increased risk for development of otitis media (OM) during childhood.

3-D Computer-Generated Models of ME and Mastoid Antrum. Levine et al[4] developed a 3-D model of the ME and mastoid antrum using serially sectioned, celloidin-embedded temporal bone slides. The sections were scanned and rendered into a 3-D image.

Pathology of Chorda Tympani Nerve in Chronic Ear Disease. Felix and Linder[5] analyzed chorda tympani nerve biopsies by light and electron microscopy. The investigators found that in the majority of the specimens, a selective degeneration of the larger myelinated fibers had occurred.

IN VIVO IMAGING STUDIES

Blevins and Carter[6] studied the utility of preoperative radiologic studies in chronic OM (COM). They concluded that imaging studies, especially computed tomography (CT), can provide information regarding the nature and extent of disease, which may not be apparent on the basis of clinical findings alone.

The aim of a study by Kew et al[7] was to assess the performance of clinical methods, including otoscopy, pure tone audiometry, and tympanometry, in diagnosing ME effusions (MEEs) by using magnetic resonance imaging (MRI) as a reference standard. They concluded that tympanometry is the most sensitive audiological test in detecting the presence of MEE. The overall accuracies of tympanometry, pure tone audiometry air-bone gap, and otologic examination were, however, not found to be significantly different by these researchers.

The purpose of a study by King et al[8] was to use multiplanar high-resolution MRI to detail the patterns of tumor spread in nasopharyngeal carcinoma and to correlate the findings with the presence of an effusion in the ME and mastoid. Their results suggest that the cause of effusion in nasopharyngeal carcinoma is multifactorial and that the invasion of the TVPM occurs in patients with effusion, suggesting a functional cause.

Swarts et al[9] studied individuals with and without a history of OM, using MRI. They showed that differences in morphometric features of the ET cartilage, including its height and cross-sectional area, existed between the individuals with no history of OM and those with persistent OM and ET dysfunction. The former group had a larger cross-sectional area, as well as a greater height.

Alper et al[10] used MRI to define in vivo the effect of experimental functional obstruction of the ET on vascular permeability and the development of MEE in cynomolgus monkeys. Their results support a causal relationship between ET dysfunction, ME underpressures, increased vascular permeability, and OM with effusion (OME).

Alper et al[11] analyzed paired results for the intensity values of the T2-weighted MRI scans and the tympanometric width measured in cynomolgus monkeys in order to correlate tympanometric gradient with measurements of effusion quantity by use of MRI during an experimental OME episode. They observed significant correlations between tympanometric width and MRI measures of effusion, confirming the high predictive value of the tympanometric width for diagnosing the presence and quantity of MEE.

Alper and Doyle[12] used cynomolgus monkeys with experimental OME to compare the diagnosis of OME made by using estimates of tympanometric gradient with that provided by simultaneous MRI of the ME. Measurements of tympanometric gradient were highly correlated with the quantity of MEE measured by MRI.

CELL CULTURE SYSTEMS AND IMMORTALIZED CELLS

In a study by Fujihara et al,[13] outgrowth cultures of human nasal polyps on coverslips were used to study the effect of nontypeable *Haemophilus influenzae* (NTHi) on upper respiratory tract mucosa by assessing ciliary morphology and ciliary beat frequency. The authors concluded that the cilio-inhibitory effects of NTHi could impair the clearance function of nasal cells and facilitate bacterial multiplication, as well as penetration of the mucociliary barrier, and thus play a major role in the pathogenesis of COM.

Schousboe et al[14] provided a quantitative description of rabbit ME epithelial cell growth in a standardized model system under controlled conditions. The results proved the method to be reproducible regarding all parameters. All parameters were without significant changes when related to DNA amount, reflecting a uniform cellular activity throughout the period.

Using specimens from the posterior-superior quadrant of the human tympanic membrane, meatal skin, retroauricular skin, and ME cholesteatoma, Koba et al[15] studied epidermal cell proliferation. They concluded that the addition of newly proliferated cells at the proliferation centers and uniform cell death cause epidermal cell migration over the tympanic membrane and ear canal. Furthermore, 5-bromo-2'-deoxyuridine (BrdU) labeling of cells at the margin of persistently perforated tympanic membranes from patients with COM revealed that at the perforation margin, the counts of BrdU-labeled cells were not higher than those of the normal tympanic membrane. In addition, the fact that few BrdU-labeled cells were observed in the lamina propria and mucosal cell layer indicates that persistent perforation of the tympanic membrane results from the failure of proliferating cells to increase at the margin of the perforation.

Minotti et al[16] determined the effects of a diminished extracellular calcium (Ca^{2+}) level on cholesteatoma migration and adhesion. When cultured specimens were exposed to low-Ca^{2+} medium, a greater than 10-fold reduction in the rate of migration was observed as compared with control values. These findings suggest that Ca^{2+}-dependent intercellular and substrate cellular adhesions are essential for cholesteatoma migration and adhesion.

Yen et al[17] investigated the ion transport mechanisms of the ME epithelium using the MESV cell line (derived from Mongolian gerbil's ME epithelial cells transformed by SV40). These investigators evaluated short-circuit current changes in response to specific stimulation of putative purinoceptors by adenosine triphosphate (ATP) and its various analogs. The fact that ATP dramatically increased the short-circuit current, while adenosine had no effect, demonstrated the presence of P2 purine receptors on these cells. The ATP-induced increase in short-circuit current was related to sodium (Na^+) transport.

Ikeda et al[18] applied *sai-rei-to*, a type of *kampo* medicine (Japanese herbal medicine), to ME epithelium cultured from Mongolian gerbils. Application of *sai-rei-to* induced an increase in the short-circuit current in the basal state. The increase in short-circuit current was almost completely inhibited by addition of diphenylamine-2-carboxylic acid, but not by amiloride, indicating enhancement of chloride (Cl^-) secretion.

The effect of chronic hypoxia on ion transport was investigated by Portier et al[19] on the MESV Mongolian gerbil ME cell line using the short-circuit current technique. The investigators concluded that this effect of oxygen (O2) on Na^+ absorption might account for 1) the presence of fluid in the ME in the course of secretory OM and 2) the beneficial effect of the ventilation tube in treating OM that allows the PO2 to rise and restores the fluid clearance.

Using the MESV Mongolian gerbil cell line, Tan et al[20] employed the short-circuit current technique to evaluate changes in ion transport induced by glucocorticoids. Administration of dexamethasone produced a dose- and time-dependent increase in the short-circuit current in MESV cells. These data demonstrate that steroids up-regulate the transepithelial Na^+ transport in the ME epithelium.

The objective of a study by Furukawa et al[21] was to investigate the effects of ATP on ion transport and Ca^{2+} mobilization in primary cultures of gerbil ME epithelium. Extracellular ATP and other nucleotides elicited a transient increase in short-circuit current in a dose-dependent manner. The authors conclude that their results are consistent with the hypothesis that extracellular ATP and other nucleotides induce Cl^- secretion across the apical membrane in cultured gerbil ME epithelium by activation of the apical Cl^- channels via multiple modes of regulation.

The aim of a study by Furukawa et al[22] was to investigate the effects of adenosine and its analogs on Cl^- secretion in primary cultures of gerbil ME epithelium. Short-circuit current, an index of transepithelial active transport, was measured on cells cultured on porous filters. Extracellular adenosine and its analogs elicited a sustained increase in short-circuit current when added to apical or basolateral surfaces. The authors concluded that extracellular adenosine and its analogs activated the cyclic adenosine monophosphate–protein kinase A system, but not intracellular Ca^{2+}-dependent mechanisms, thereby leading to Cl^- secretion.

Li et al[23] evaluated the role of ME epithelial cells in expressing inducible nitric oxide synthase (iNOS) and the regulation of the enzyme by cytokines. They found that treatment of primary rat ME epithelial cells with tumor necrosis factor α (TNF-α) or interleukin (IL)–1β resulted in an increase in iNOS messenger RNA (mRNA) and protein.

St Geme et al[24] studied the role of HMW1 (high–molecular weight 1) and HMW2 adhesins in the interaction of NTHi with epithelial cells. Their results suggest that phase variations allow NTHi to survive in diverse environments, including the nasopharynx early in the infection and the ME later.

DeFiore-Hyrmer and Bakaletz[25] examined regions of NTHi adherence to cultured chinchilla ME epithelial cells by triple immunofluorescence labeling and confocal microscopy. They observed that the nucleation of actin in the area underlying adherent bacterial cells was coincident with areas of phosphorylated tyrosine. Their results suggest that a signaling event in which tyrosine phosphorylation occurred was coincident with both NTHi adherence and actin nucleation.

Rich et al[26] investigated the effect of products of clinical isolates of NTHi, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* on the migration of human neutrophils. All 3 bacteria inhibited neutrophil chemotaxis, and the results suggested that the activity was present in the low–molecular weight fractions, the most active of which was below 1,000 d.

Using an in vitro model system consisting of human lung epithelial cells (NCI-H292) cultured on permeable supports, van Schilfgaarde et al[27] examined the effects of long-term association of NTHi with these cells. The authors concluded that penetration of NTHi between epithelial cells results in protection for the bacterium from both antibiotics and antibodies and may thereby contribute to the persistence of this microorganism in chronic disease.

In an extension of the earlier study, van Schilfgaarde et al[28] cloned genes of NTHi involved in the penetration of these bacteria between human lung epithelial cells in vitro. Two clones were identified that contained open reading frames (ORFs) homologous to H10636 to H10641 of *H influenzae* strain Rd and that were associated with increased penetration of the human bronchial epithelial cell line NCI-H292. The authors conclude that the protein encoded by ORF H10638 may function as a paracytin, while that encoded by H10636 may have an auxiliary function.

Human ME epithelial cells were cultured by Nell et al[29] and exposed to endotoxin. The investigators concluded that endotoxin was an important factor in the disturbance of the morphology of the ME epithelium, and may lead to COM with effusion.

Human ME mucosa was explanted and cultured by Buchman and Fregien[30] and exposed to influenza A virus. These investigators showed that human ME mucosa grown over short periods retains characteristics of epithelial cells, while long-term propagation results in changes consistent with either fibroblast overgrowth or cellular dedifferentiation. Moreover, exposure to a human influenza A virus known to cause OM results in cytopathic effects, as well as immunocytochemical and molecular evidence of cellular infection.

In another study by Buchman and Fregien[31] using the same primary human ME epithelial cell culture system, these investigators have shown that human ME epithelial cells are capable of supporting retroviral-mediated gene transfer.

Cell Lines. Several ME cell lines have been successfully established from the rat, gerbil, chinchilla, and human ME epithelial cells. A chinchilla cell line was made by Jin et al[32] using the adeno12-SV40 hybrid virus. A rat ME epithelial cell line, displaying epithelial cell characteristics, was made by Ueyama et al,[33] using the adeno12-SV40 hybrid virus as well. A human ME epithelial cell line was derived by Y. M. Chun et al (unpublished observations) by expressing the E6/E7 gene products of the human papillomavirus (HPV) in the primary cells. Chun et al[34] also used the rat ME epithelial cell line, as well as a rat ET epithelial cell line, to investigate the expression of the mucins (Muc1, Muc2, and Muc5AC) and lysozyme.

Entry of S pneumoniae *Into Epithelial Cells.* Several recent studies have convincingly shown that *S pneumoniae* and NTHi can be internalized by the intact respiratory mucosal epithelial cells.[35,36] After the initial binding of *S pneumoniae* to host cell surface membranes, the bacteria can be found within membrane-bound vacuoles that initially resemble classic phagosomes.[35] Once inside cells, the bacteria seem to undergo 1 of 3 fates: 1) they are killed within the vacuole; 2) they are recycled within a vacuole back to the apical cell surface; or 3) they are transcytosed through the cell in vacuoles that fuse with the basolateral surface, thus releasing the bacteria outside the cell. It is this last fate that suggests an explanation for how this organism may be able to cross the blood-brain barrier and cause meningitis.[37]

Entry of NTHi Into Epithelial Cells. Although NTHi is not generally considered to be an intracellular pathogen, the bacterium has been shown to be able to enter the host cell, presumably to evade the local immune response. Using in situ hybridization of a fluorescein-labeled 16S ribosomal RNA-targeted oligonucleotide probe, Forsgren et al[38] showed that NTHi can reside intracellularly in human adenoid tissue. The finding of widespread presence of NTHi in cells of which some strongly expressed the CD14 marker of the monocyte/macrophage lineage may correspond to an important aspect of the colonization mechanisms whereby NTHi persists in the nasopharynx in children.

Apicella et al[39] studied the interaction of NTHi strain 2019 with epithelial cells, using electron and confocal microscopy. The investigators established primary cultures of human airway epithelial cells on collagen-coated glass coverslips or on semipermeable membranes at the air-liquid interface. The results suggest that NTHi binds to nonciliated cells and that the infection causes actin polymerization with an increase in cortical actin, as well as an increase of actin strands around the bacteria. The bacteria were surrounded by lamellipodia and microvilli and were also present in vacuoles inside the cells. The NTHi appeared to be entering the cells by macropinocytosis.

Ketterer et al[40] initiated studies to explore the interaction of the NTHi strain 2019 with primary human airway epithelial cells by electron and confocal microscopy. The surface of infected cells showed evidence of cytoskeletal rearrangements manifested by microvilli and lamellipodia extending toward and engaging bacteria. Confocal microscopic analysis demonstrated that infection induced actin polymerization, with an increase in cortical actin, as well as evidence of actin strands around the bacteria. Transmission electron microscopic analysis showed lamellipodia and microvilli surrounding organisms, as well as organisms adherent to the cell surface. These studies also demonstrated the presence of bacteria within vacuoles inside of airway cells. Confocal microscopic studies indicated that NTHi was entering cells by the process of macropinocytosis. These studies indicate that NTHi can initiate cytoskeletal rearrangement within human airway epithelium, resulting in internalization of the bacteria within nonciliated human airway epithelial cells by the process of macropinocytosis. In addition to the above observations, wild-type NTHi strains have been found, in vitro, to adhere to, enter, and persist in Chang epithelial cells and in chinchilla respiratory tract epithelial cells.[36]

Mastoid Pneumatization. The objective of a study by Aoki et al[41] was to investigate whether the growth of the mastoid air cells and the ME transmucosal gas exchange function in patients with OME was affected by the histopathologic changes in ME mucosa, and whether these two factors were changed by the treatment of OME. The investigators observed that the suppression of the cell growth area was greater and the ME total pressure was lower in cases with numerous mucosal lesions.

Hasebe et al[42] studied the outcome of tympanostomy tube insertion on pneumatization in children with OME. They conclude that mastoid pneumatization can improve in children with OME and that insertion of tympanostomy tubes should be done before age 9.

PATHOGENESIS

Free radicals participate in the development of disease under inflammatory conditions. Lipid peroxides such as malondialdehyde are regarded as markers of cell membrane damage by oxidative stresses. It has been shown that lipid peroxidation is increased in acute OM (AOM) in guinea pigs, implying a role for free radicals as contributors to inflammation.[43]

The aim of the study by Takoudes and Haddad[44] was to examine human ME fluid for lipid hydroperoxides as evidence of free radical damage. Their results demonstrated the presence of lipid hydroperoxide in all 3 types of ME fluid (serous, mucoid, and purulent). Additionally, there was a statistically significant elevation of total lipid hydroperoxide content in mucoid effusions as compared with serous effusions.

John et al[45] determined the concentrations of nitric oxide (NO) in MEEs. Effusions were collected from patients and classified as serous, mucoid, or purulent. Effusions were also collected from chinchillas inoculated with lipopolysaccharide (LPS) into the ME. Analysis of the samples revealed that all contained high concentrations of NO, indicating that NO production by NO synthase — in particular, iNOS — may be important in the pathogenesis of OM.

Deficiencies in the collectins can result in susceptibility to infections, as evidenced by studies of the association of mannan-binding lectin (MBL) with infections. Studies conducted by Summerfield et al[46] suggest that specific mutations of MBL may indeed be associated with an increased incidence of OM in at least some children. Additionally, Babovic-Vuksanovic et al,[47] Sumiya and Summerfield,[48] and Kakkanaiah et al[49] have shown that low serum levels of MBL are associated with recurrent infections in adulthood and early childhood, as well as autoimmunity. Other studies, however, have suggested that children with homozygosity for the normal MBL genotype did not have more episodes of AOM or recurrent AOM, and do not have the first episode of AOM at a younger age.[50]

Serum opsonization of yeast (*Saccharomyces*) was investigated by Tezcan et al[51] in children with acute purulent OM between 6 and 48 months of age and in an age-matched control group. The authors suggested that their results indirectly supported the role of opsinins such as MBL, whose defects have been correlated with propensity to infections, in OM.

Another family of molecules of innate immunity whose expression has been studied in the ear are the defensins. Boe et al[52] investigated the expression of the mRNA for human β-defensin 1 (HBD-1) in the tympanic membrane and adjacent areas. Their results indicated that HBD-1 was expressed in the pars tensa, pars flaccida, and meatal skin. The transcripts were localized to the keratinocytes of the epidermal layer of these tissues, but could not be detected in the tympanal epithelium of the eardrum. The authors concluded that HBD-1 may be involved in the innate defenses of the external ear canal.

Studies by Lim et al[53] have shown that molecules of the innate immune system, including β-defensins 1 and 2, surfactant proteins A and D (SP-A, SP-D), lysozyme, and lactoferrin, are expressed by ME epithelial cells, and thus may play an important role in the protection of the tubotympanum.

IMMUNOHISTOCHEMISTRY AND HISTOLOGY

Hurst[54] ascertained the role of allergy, as defined by skin testing and histochemical markers, in the pathogenesis of OME. The relationship between allergy and OME was corroborated clinically in 89% of patients and was also substantiated by elevated levels of effusion eosinophil cationic protein (ECP) in 87.5% of OME patients. Histologically, polyclonal antibody staining for ECP demonstrated the presence of eosinophils in ME mucosal biopsy specimens. This study suggests that OME is a sign of allergic inflammation in the ME that is associated with an increase in eosinophils and a concomitant release of ECP into the effusion in individuals with allergy demonstrated by skin testing.

The majority of patients with OME and atopy have been shown to have elevated levels of ECP in their ME fluid. The purpose of the study by Amin et al[55] was to investigate the feasibility of a quantitative determination of eosinophils and neutrophils in the ME lining by specific immunocytochemical markers, in order to study the extent of the involvement of these cells in patients with OME. The investigators found that eosinophils and neutrophils were present in the subepithelial connective tissue and in the mucus blanket in the ME of patients with OME in significantly higher numbers than in the control group.

Hurst and Fredens[56] evaluated patients with persistent MEE and allergy confirmed by skin testing for eosinophils by histochemical staining of ME mucosal biopsy specimens for ECP. They found that the majority of the patients with OME had ECP containing eosinophils and degranulated ECP material in the stroma of their mucosal biopsy specimens. Positive and negative biopsy findings correlated directly with respective high and low effusion levels of ECP.

In another study, Hurst et al[57] analyzed chronic MEE of patients for the presence of tryptase, an indicator of mast cell activity. The investigators found that the majority of patients had extensive activation of mast cells in their MEs and had elevated levels of effusion ECP. The presence of mast cells, indicators of a Th2-driven immune response, in the majority of ears that have chronic effusion suggests that the inflammation within the ME of most OME patients may be allergic in nature.

Matsune et al[58] investigated the presence of mucosal lymphoid follicles with germinal centers, so-called mucosa-associated lymphoid tissue (MALT), in the ET and ME in temporal bones containing the entire ET obtained from children. Greater numbers of MALTs were found in specimens from children with OM than from those without OM. These results support the hypothesis that persistent and recurrent inflammation in the nasopharynx spreads through the ET to play a role in the pathogenesis of COM in children.

Sudo and Sando[59] examined histologic sections of human temporal bone with the ET attached to investigate the changes that occur in folding of the ET as it develops postnatally. Their results suggest that folding of the ET is important to its function in infants and children.

Jecker et al[60] studied the ME and ET mucosa as a unit in young rats with respect to the composition of immunocompetent cells before and after ME infection. They found macrophages and dendritic cells throughout the ME mucosa, whereas only a few NK cells and T and B lymphocytes, and almost no granulocytes, were detected. In the ET mucosa, immunocompetent cells — with the exception of dendritic cells and macrophages — were rarely seen. After induction of OM by severing the soft palate, immunocompetent cells increased in both the ME and ET mucosa, but were almost absent from the area of the tubal orifice to the ME.

Ganbo et al[61] carried out immunohistochemical analyses to investigate the distribution of lymphocytes and macrophages in routine human temporal bone sections obtained from a subject with acute suppurative OM. Their results demonstrated a nonuniform distribution of T lymphocytes, B lymphocytes, and macrophages in the mucosa of the ET, tympanic cavity, and mastoid air cells.

Forseni et al[62] evaluated the inflammatory stages that may lead to tympanosclerosis or myringosclerosis, using Sprague-Dawley rats inoculated transtympanically with pneumococcus type 3. Groups of rats were killed at various times after inoculation. Sections from the tympanic membrane were stained immunohistochemically. The results showed that macrophages, T cells, and B cells were presented time-dependently in the acute inflammatory response in AOM. The investigators concluded that macrophages were the first cells to invade the tissue after AOM induction.

Parks et al[63] studied the distribution and relative abundance of the antioxidant enzyme catalase in the ME of guinea pigs in an effort to elucidate the role free radicals play in the pathogenesis of OM. Catalase was localized immunohistochemically to the epithelium and had scant distribution in the submucosa — similar to the pattern seen in the control ears.

Takoudes and Haddad[64] conducted a study to examine the ME fluid in a guinea pig model of AOM for evidence of elevated lipid peroxide as a marker of free radical damage. Histologic analysis was performed to assess the degree of tissue damage. Their results indicated an increased absolute level of lipid peroxide, and they found a correlation between mucosal damage and the extent of lipid peroxides in the fluid.

Studies of Forseni et al[65] were aimed at immunohistochemical analysis of the expression of iNOS in an *S pneumoniae*–induced rat model of AOM. They showed that iNOS protein was strongly expressed in the ME mucosa and in the tympanic membrane, as well as in the inner ear.

Using lectin histochemistry, Sone et al[66] studied alterations in glycoconjugates in normal temporal bones, temporal bones with mucoid OM, and those with serous OM. Their results demonstrated that the ETs from cases with serous OM or mucoid OM had alterations in glycoconjugate expression in cilia and goblet cells, which may reflect a disorder of carbohydrate metabolism in OM, especially serous OM.

Jung et al[67] investigated the expression of vascular endothelial growth factor (VEGF) in both endotoxin-induced OME of the rat and in human OM. The investigators instilled endotoxin or saline solution into the ME of rats and analyzed the expression of VEGF mRNA and VEGF protein using semiquantitative reverse transcriptase–polymerase chain reaction and immunohistochemistry. Their results suggest that VEGF may be responsible for increased vascular permeability and endothelial cell growth in OME and that VEGF may play a significant role in the pathogenesis of OME.

Nagaraj and Linthicum[68] used immunohistochemical analysis of celloidin-embedded temporal bone samples to demonstrate the presence of catecholaminergic nerve fibers in the human ME mucous membranes that may play an important role in the pathogenesis of MEE. The investigators concluded that these catecholaminergic nerve fibers, as elsewhere in the body, may exert a direct influence on the ME mucosal blood vessels and suggested that the effusion into the ME space was an active, rather than a passive, process. Moreover, they suggested that cholinergic-sympathetic nerves might exist in the human ME mucous membrane, and that these autonomic nerves, in conjunction with the neuropeptides, might play an active role in the pathogenesis of human MEE.

Goycoolea and Muchow[69] studied by electron microscopy the mechanisms of new gland formation in human ME mucoperiosteal tissue samples obtained from patients with a history of OM. They observed that in the areas of inflammation, the mucoperiosteum acquired polypoidal characteristics. The epithelium also became taller, and cells developed increased secretory activity. Moreover, the invaginated portions of the polypoidal formations were shown to be lined with secretory epithelium. The authors concluded that gland formation may occur in the invaginated region by fusion of epithelial ruptures and the budding off of dense cellular foci from the epithelium.

The calcification of the ET cartilage and the atrophy of the TVPM were studied by Takasaki et al[70] in normal human temporal bones obtained from individuals with ages ranging from 2 days to 88 years. The investigators found a statistically significant correlation between the number of the calcified cells and aging ($p < .001$). A statistically significant correlation was also found between the degree of atrophy of the TVPM and aging ($p < .001$). The authors concluded that calcification of the ET cartilage and atrophy of the TVPM are closely associated with aging. Therefore, it is suggested that these two findings may be a predisposing factor for ET dysfunction in elderly adults.

IN SITU HYBRIDIZATION

Jin et al[32] used PCR and in situ hybridization for the detection of HPV in screening cases of squamous cell carcinoma of the ME from archival material. The majority of the samples were found to have HPV DNA.

Watanaabe et al[71] induced OME in BALB/c mice by intratympanic inoculation of NTHi lipooligosaccharide or murine Il-1β. The mRNA for IL-1β was localized by in situ hybridization. Both groups of mice had effusion and were histologically similar. The IL-1β mRNA was shown to be expressed in the ME epithelium.

INNER EAR TOXICITY AND OTITIS MEDIA

Jung et al[72] studied the effect of leukotriene (LT) inhibitor (L-663,536) on salicylate-induced morphological changes of isolated cochlear outer hair cells. They showed that pretreatment with L-663,536 prevented salicylate-induced hearing loss. Their results suggest that arachidonic acid metabolites, especially LTs, seem to play an important role in the pathogenesis of salicylate ototoxicity.

Ichimiya et al[73] induced labyrinthitis by inoculation of keyhole limpet hemocyanin into the scala tympani of systemically sensitized guinea pigs. Their results suggested that inflammatory cells and blood constituents could extravasate into the cochlea from blood vessels and that fibrocyte damage in the spiral ligament could cause cochlear dysfunction.

Engel et al[74] studied the passage of radioiodinated streptolysin-O and albumin through the round window membrane. They found that when applied to the ME, streptolysin-O became quantitatively entrapped in this compartment and no passage to the cochlea occurred. The authors proposed that the passage of noxious macromolecules, such as proteases, from a purulent MEE may be facilitated by pore-forming toxins, resulting in cochlear damage and sensorineural hearing loss.

Ichimiya et al[75] investigated the cochlear influence of pneumococcal OM in mice in order to identify damaged regions causing cochlear malfunction. Otitis media was induced in all of the animals, and some showed inflammatory cells in the cochlea. Immunohistochemistry showed the presence of fibrinogen in the cochlea, mainly in the lower portion of the spiral ligament and in the spiral limbus. Immunostaining for connexin 26 was decreased in the spiral ligament and was accompanied by marked fibrinogen staining. Immunostaining for sodium-potassium-adenosine triphosphatase in the stria vascularis and in the type II fibrocytes of the spiral ligament was not affected significantly.

Juhn et al[76] assessed the effect of the administration of the NO donor sodium nitriprusside, through the round window membrane, on cochlear function. The investigators observed a concentration-dependent auditory brain stem response threshold shift after sodium nitriprusside instillation. This drop in hearing was concluded to be the result of damage to the hair cells and supporting cells caused by NO.

Hess et al[77] studied the expression of iNOS (NOS II) in the cochlea of guinea pigs after intratympanic endotoxin and TNF-α treatment. Expression of iNOS in the cochlea was observed in the wall of blood vessels of the spiral ligament and the modiolus, in supporting cells of the organ of Corti, in the limbus, in nerve fibers, and in a part of the perikarya of the spiral ganglion after LPS/TNF-α treatment. They concluded that the high amounts of iNOS-generated NO could have dysregulative and neurotoxic effects on the inner ear during bacterial and viral infections of the middle and inner ear.

In order to determine whether the bacterial toxins associated with OM could induce morphological changes in the organ of Corti, Cook et al[78] inoculated the ME cavities of healthy guinea pigs with *S pneumoniae* and examined the organ of Corti histologically at several time points after inoculation. Their results suggested that bacterial OM can produce cytotoxic changes in the cochlea.

Stenqvist et al[79] instilled *Pseudomonas aeruginosa* exotoxin A into the ME cavity of rats. Morphological changes of the mucosa were analyzed by light and transmission electron microscopy. Their findings showed that denuding of the lamina propria may facilitate the penetration of toxin into the labyrinth, thus explaining subsequent inner ear damage.

SYNDROMES ASSOCIATED WITH OTITIS MEDIA

Ganbo et al[80] studied the inflammatory response to COM in DiGeorge syndrome. They conducted immunohistochemical analyses on archival celloidin-embedded human temporal bone sections from an 8-month-old boy with COM and DiGeorge syndrome. B lymphocytes and macrophages were observed predominantly within the ME mucosa, and T lymphocytes were rare. Expression of intercellular adhesion molecule 1 (ICAM-1) was seen in the vascular endothelium of the lamina propria, as well as infiltrating mononuclear cells. The results suggest that the expression of ICAM-1 can be induced in the ME during OM, even in a cell-mediated immunodeficiency disorder such as DiGeorge syndrome.

Handzic-Cuk et al[81] examined the characteristics of mastoid pneumatization in Pierre Robin syndrome (PRS) and non-PRS cleft palate populations in relation to age. They found that the median area of mastoid bone pneumatization (MBP) in PRS patients was significantly lower than that in non-PRS cleft patients. The MBP area was also smaller than in the patients with unilateral cleft lip and palate and isolated cleft palate. The MBP area did not change significantly with age in PRS or in the patients with bilateral cleft lip and palate, but it did increase significantly in the isolated cleft palate group. The investigators concluded that pneumatization in all cases increased with age, but this increase was statistically significant only in patients with isolated cleft palate. The PRS patients had a smaller area of MBP than did the non-PRS cleft palate population. The extent of MBP in PRS patients did not correlate with age, because of the negative influence of the mandibular hypoplasia and glossoptosis present.

ANIMAL MODELS

Streptococcus pneumoniae. Alper et al[82] evaluated the efficacy of clarithromycin treatment of AOM caused by infection with penicillin-susceptible, -intermediate, and -resistant *S pneumoniae* in the chinchilla. These results support the use of the chinchilla to evaluate drug efficacy in the treatment of AOM and show clarithromycin to be effective in sterilizing the MEs of animals challenged with penicillin-susceptible, -intermediate, and -resistant strains of *S pneumoniae.*

Barry et al[83] used a gerbil model of OM to assess the efficacy of single-dose ceftriaxone sodium against 3 *S pneumoniae* strains highly resistant to penicillin and with various susceptibilities to ceftriaxone. Their results demonstrate the efficacy of ceftriaxone against experimental cephalosporin-resistant pneumococcal OM and provide a basis for the clinical use of single-dose ceftriaxone against pneumococcal OM.

Magnuson et al[84] studied the course of healing of the rat tympanic membrane when myringotomy was performed during AOM caused by *S pneumoniae.* Their results suggest that a combination of bacterial infection and myringotomy causes long-lasting changes in tympanic membrane structure. They conclude that the impaired structure of the connective tissue could be of importance in chronic ME disease as a presumptive site for retraction and perforation of the tympanic membrane.

Mattsson et al[85] induced purulent OM in rats by inoculating *S pneumoniae* into the ME cavity of rats. Myringotomy was next performed on the animals, and the tympanic membranes were examined both otomicroscopically and histologically. On histologic examination, it was found that the frequency and extension of sclerotic lesions in the tympanic membrane of both the noninfected myringotomized animals and the myringotomized animals with AOM were similar, and that the nonmyringotomized rats with AOM were free of sclerotic lesions.

Cayé-Thomasen et al[86] inoculated pneumococci into the ME bulla of rats and examined the ME mucosae by light microscopy. They concluded that ME adhesion is a pathological phenomenon caused by infection and proposed a 6-stage hypothesis of pathogenesis: 1) localized epithelial rupture, 2) prolapse of subepithelial tissue, 3) epithelialization of the prolapse, resulting in a polypous, fold-like prominence, 4) growth and elongation of the prominence, 5) fusion of the end/tip of the prominence with another part of the mucosa, and 6) formation of an adhesion.

The objective of another study by Cayé-Thomasen et al[87] was to assess the dynamics of bone modeling in a rat model of acute pneumococcal OM, studied longitudinally from day 1 through 6 months after inoculation. Their results revealed initial bone resorption, followed by increasing apposition of new bone in the ME cavity, initiated at the outer periosteum. Bone thickness in 4 anatomically distinct locations peaked at 3 months and was still high 6 months after the acute incident. The authors concluded that their findings support the existence of a perilymphatic barrier of specialized bone, and suggest that even a single episode of acute infection may alter properties of ossicular chain conduction. Furthermore, they believed their results might support the environmental theory of mastoid pneumatization, claiming inflammatory disease as the cause of a sclerotic mastoid process.

To investigate the effect of penicillin on the pathological bone remodeling in AOM, Cayé-Thomasen and Tos[69] inoculated rats with pneumococci and, after oral administration of penicillin V, sacrificed them at several time points. Bone histomorphology was registered, and the thickness was measured. The authors concluded that penicillin reduces new bone formation in AOM, leaving other features of histomorphology unchanged.

Johnson et al[88] gave ICR mice transtympanic injections of human IL-8, heat-killed *S pneumoniae*, or normal saline. The temporal bones were then studied. Their results demonstrated that IL-8 is a potent inducer of ME inflammation and support the concept that IL-8 may be one of the key cytokines responsible for the leukocyte accumulation and activation seen in OM.

To better characterize the mechanism of free radical damage in AOM, Takoudes and Haddad[89] studied the production of hydrogen peroxide by *S pneumoniae*. Guinea pigs were injected transtympanically with bacteria in the left ME and with sterile saline in the right ME. Their results indicated higher hydrogen peroxide levels in infected versus control ME fluid.

Haddad[43] evaluated whether lipid peroxide continues to be present for a period of time after ME infection in guinea pigs by *S pneumoniae*. Analysis of histologic sections of ME mucosa confirmed the presence of inflammation. A statistically significant increase in lipid peroxide was seen at each time point studied, compared with controls, thus demonstrating that lipid peroxide may contribute to ME inflammation.

In another study, Haddad et al[90] examined the effect of lazaroid U-74389G, a 21-aminosteroid, on *S pneumoniae*–induced AOM in guinea pigs. Their results suggested that lazaroid may reduce lipid peroxide in the ME at an early stage of AOM.

To determine the role of inflammatory products of phospholipid metabolism in AOM, Diven et al[91] infected chinchillas with *S pneumoniae*. The animals were randomly assigned to 1 of 4 treatment groups receiving intramuscular ampicillin sodium (control) or intramuscular ampicillin plus receptor blockers of platelet activating factor (WEB 2086), of LT (MK 571), or of thromboxane A2 (GR 32191B). Their results showed that the addition of a receptor blocker for either platelet activating factor and/or thromboxane to ampicillin in the treatment of AOM reduces mucosal inflammation and decreases the production of other inflammatory chemicals. According to the authors, the failure of a receptor blocker of LTs to moderate disease expression suggests either a less important role for these chemicals in AOM or an insufficient bioavailability of the specific MK 571 inhibitor. These results confirm that platelet activating factor and thromboxane are active mediators of inflammation in AOM.

Van der Ven et al[92] developed a new rat model of OM caused by *S pneumoniae*. To do this, they impaired the mucociliary clearance mechanisms of the ET by infusing histamine into the tympanic cavity on 2 consecutive days before intranasal inoculation of the bacteria. They concluded that they had developed a new animal model for pneumococcal OM that was suitable for the evaluation of vaccine candidates.

The studies by Don et al[93] were aimed at developing an animal model of acute and chronic suppurative OM. They inoculated MEs of chinchillas with a low-virulence strain of *S pneumoniae* (group 1), *Staphylococcus aureus* (group 2), heat-killed *P aeruginosa* (group 3), LPS from *Klebsiella pneumoniae* (group 4), croton oil (a strong irritant; group 5), or glycerin (induces ME inflammation; group 6). All the animals developed otorrhea and ME inflammation, but groups 1, 3, and 5 had the most robust reactions.

Sato et al[94] characterized ME inflammatory responses in the chinchilla OM model after injecting viable pneumococci into the ME. Middle ear fluid concentrations of IL-1β, IL-6, IL-8, and TNF-α were measured. The IL-6, IL-8, and TNF-α concentrations, but not IL-1β concentrations, correlated significantly with total inflammatory cell numbers in ME fluid, and all 4 cytokines correlated significantly with ME fluid neutrophil concentration.

Streptococcus pyogenes. Fulghum and Brown[95] used guinea pig and chinchilla models to determine whether highly purified streptococcal cell wall peptidoglycan-polysaccharide isolated from group A *Streptococcus pyogenes* could itself cause inflammation in the ME. The authors concluded that bacterial cell wall components themselves could produce histologic evidence of acute and chronic inflammation in the ME. Thus, their data support earlier suggestions that when bacterial cell wall components are trapped in the ME, they can initiate or extend inflammation, thus predisposing to either chronic or recurrent OM.

Haemophilus influenzae. Magnuson et al[96] inoculated NTHi and encapsulated *H influenzae* type b (Hib) into the middle ears of Sprague-Dawley rats. Tympanic membrane status was assessed otomicroscopically, and specimens from various ME areas were prepared for light microscopy at various times. The ME mucosa of NTHi-infected ears initially showed vigorous activity among the goblet cells, but the mucosa normalized after the acute phase. By contrast, Hib induced prominent changes in the ME mucosa. Initially, no goblet cell granules or ciliated cells could be observed in the mucosa. Later, large, active goblet cells were seen in the epithelium. Glands appeared beneath the mucosa that persisted as streaks of epithelial cells throughout the study period. The findings showed that NTHi and Hib both induced AOM, but with differing clinical courses, and affected different targets in the ME.

Cayé-Thomasen et al[97] inoculated the MEs of rats with Hib to determine the effect of the bacteria on mucosal secretory capacity. Compared with that of normal MEs, the goblet cell density was significantly increased in the treated rats.

Cayé-Thomasen et al[98] used a rat model to determine whether inoculation of the ME cavity with NTHi would lead to an increase in goblet cell density in the ME mucosa, as had already been shown to occur after inoculation with *S pneumoniae*. These investigators found that goblet cell density was significantly increased in all locations and at all time points for infected rats. Moreover, the induced increase in goblet cell density was greater than that which had been reported after inoculation with *S pneumoniae*. The authors concluded that NTHi-induced AOM is followed by an increase in mucosal secretory capacity, which probably predisposes to a subsequent secretory OM.

DeMaria et al[99] used lipooligosaccharide-deficient NTHi strains to test the ability of these bacteria to colonize the nasopharynx and to induce OM subsequent to transbullar inoculation in the chinchilla model. Their nasopharyngeal colonization data indicated that the parental strain and the mutants were able to colonize the nasopharynx and exhibited comparable clearance kinetics. The mutants, however, required a much higher dose and did not exhibit evidence of sustained multiplication.

Loosmore et al[100] tested the protective effect of recombinant *H influenzae* HtrA (high temperature requirement A) protein in an active chinchilla model of OM. Ablation of the endogenous serine protease activity of wild-type HtrA did not decrease immunogenicity, and the mutant was found to be protective in the OM model. The authors concluded that the mutant HtrA may be a good candidate for a vaccine against Hib disease and OM.

Bakaletz et al[101] conducted 3 separate studies — 2 involving active immunization regimens and 1 involving a passive transfer protocol — in order to initially screen and assess several NTHi outer membrane proteins or their derivatives for their relative protective efficacy in a chinchilla model of OM. They tested P5 fimbrin, the synthetic chimeric peptide LB1 (derived from P5 fimbrin), lipoprotein D, nonacylated lipoprotein D, a recombinant peptide designated LPD-LB1(f)2,1,3, and P6. The authors concluded that there is merit in further development of LB1 and LPD-LB1(f)2,1,3 as components of vaccines for OM.

Ponte et al[102] used a gerbil model of OM induced by a β-lactamase–producing isolate of NTHi to assess the in vivo efficacy of co-amoxiclav (amoxicillin–clavulanic acid) and cefuroxime. They concluded that for OME, antibiotics should be administered in high doses and in short courses.

Sato et al[103] characterized changes in the levels of IL-1β, IL-6, IL-8, and TNF-α in ME fluid of guinea pigs in response to administration of nonviable *H influenzae* into the ME. Their results suggest that ME mucosa produces IL-1β and TNF-α first, followed by accumulation of inflammatory cells and the subsequent production of IL-6 and IL-8.

Moraxella catarrhalis. Fulghum and Marrow[104] studied the effect of inoculating viable and heat-killed suspensions of *M catarrhalis* into the ME cavity of gerbils and chinchillas. The authors concluded 1) that gerbils and chinchillas are susceptible to a self-limited inflammation caused by *M catarrhalis*; 2) that no infection occurs, since viable bacteria could not be recovered from ME aspirates; and 3) that viable cells produced more severe inflammation than heat-killed cells.

To determine whether *M catarrhalis* had pathogenicity in the ME, Sato[105] inoculated heat-killed *M catarrhalis* into the ME bullae of guinea pigs and studied the inflammatory response. He concluded that nonviable *M catarrhalis* induced ME inflammation and mucoperiosteal histopathology, and that these might be caused by direct injury of the nonviable bacteria (eg, lipooligosaccharide or outer membrane proteins) and metabolic products of inflammatory cells.

To study the effects of viable and heat-killed *M catarrhalis* on the ME mucosa, and to evaluate the protection after whole-cell immunizations,Westman et al[106] challenged Sprague-Dawley rats with 4 different *M catarrhalis* strains, followed by a rechallenge. The authors observed that only viable bacteria at a high concentration induced purulent OM that was culture-positive on day 4. The infection lasted about 8 days. Structurally, the effects of the heat-killed bacteria were less pronounced in the early phase as compared to the viable bacteria, but they were similar at the end of the experiment at 6 months. The authors concluded that the rat may be useful for the study of different aspects of *M catarrhalis* OM.

Staphylococcus aureus. Sutbeyaz et al[107] studied the anti-inflammatory effects of SC-41930 (a specific LTB4 receptor antagonist) in a guinea pig model of OM induced by ME inoculation of killed *S aureus*. Myeloperoxidase activity in the SC-41930–treated group was found to be significantly lower than that in the control group. Histopathology of temporal bones indicated decreased inflammation in the treated group as compared to the controls. In addition, ME fluids were absent in 4 of 6 treated animals. These results demonstrate that SC-41930 can produce significant anti-inflammatory effects in this model of OM.

Pseudomonas aeruginosa. The purpose of the studies by Dohar et al[108] was to determine the safety and efficacy of topical ciprofloxacin hydrochloride in the treatment of experimental chronic suppurative OM caused by *P aeruginosa* infection in cynomolgus monkeys. Histopathologic data revealed no statistically significant difference in the amount of outer hair cell loss for the ciprofloxacin group as compared to the control group. The authors concluded that in this animal model, topical ciprofloxacin is not ototoxic and is effective in sterilizing the otorrhea, but does not promote resolution of the drainage.

Lipopolysaccharide and Other Agents. Enomoto et al[109] examined the effect of erythromycin on the expression of neutrophil adhesion molecules such as L-selectin and Mac-1, in order to elucidate its possible mechanism of action. Furthermore, the investigators examined production of LTB4, LTC4, and prostaglandin E2 (PGE2) in rat OME induced by injection of an LPS. Erythromycin down-regulated L-selectin expression and inhibited up-regulation of Mac-1 expression on peripheral blood neutrophils. It also inhibited the accumulation of inflammatory cells such as neutrophils and macrophages in MEE. Furthermore, erythromycin suppressed the exudation of plasma protein and the production of LTB4, LTC4, and PGE2 in OME. These results suggest that erythromycin may exert an anti-inflammatory effect on MEE through suppression of leukocyte accumulation in the ME by affecting the expression of adhesion molecules on leukocytes and inhibiting the production of arachidonic acid metabolites.

Yoshioka et al[110] investigated the gene expression and production of GRO/CINC-1 protein in ME lavage of LPS-inoculated rats. The levels of GRO/CINC-1 protein in the ME lavage increased after LPS stimulation. Topical corticosteroid perfusion in the ME after LPS stimulation significantly reduced the production of GRO/CINC-1.

Darrow and Keithley[111] investigated the efficacy of polymyxin B, a polypeptide antibiotic known to bind to and inactivate endotoxin, as a modulator of the inflammatory response to endotoxin in the ME. In a guinea pig model, cellular infiltrate, effusion volume, and mucosal edema in response to endotoxin were reduced in the presence of polymyxin B. These results suggest a potential role for the use of polymyxin B in the management of MEE.

Mitchell et al[112] used the C3H/HeJ and C3H/HeSnJ strains of mice and evaluated them for incidence of spontaneous ME disease at various ages. None of the LPS-responsive C3H/HeSnJ mice demonstrated ME disease. However, ME disease was found in one third of the C3H/HeJ mice. The investigators concluded that the reduced LPS responsiveness of C3H/HeJ mice makes them less capable of reacting immunologically to bacterial infection and presumably underlies the failure to clear ME disease.

To study the actions of glucocorticoids on the development and progression of endotoxin-mediated OME, Baggett et al[113] exposed the MEs of Sprague-Dawley rats to Krebs-Ringer solution or LPS. Among the group that received LPS, some rats were randomly selected to receive dexamethasone. The investigators concluded that dexamethasone was effective in inhibiting the development of endotoxin-induced

OME.

Sugiura et al[114] studied the effect of roxithromycin on endotoxin-induced OME in the guinea pig. The authors concluded that the clinical application of roxithromycin could be an effective measure to prevent the occurrence of OME and also the recurrence of the disease, especially in OME-prone individuals.

N-nitro-L-arginine methyl ester (L-NAME), a competitive inhibitor of NO synthase, was used by Rose et al[115] to investigate the role of NO in mucin secretion by the ME epithelium during experimental endotoxin-induced OME in rats. The volume of effusion and the quantity of mucin collected were significantly greater in LPS-exposed ears than in controls. The LPS-induced production of mucin and mucous cell hyperplasia, however, was inhibited in ears treated with LPS and L-NAME; this finding suggests that NO is a mediator in the pathway of mucin secretion in chronic OME.

Maeda et al[116] developed a murine model of OME using BALB/c mice with a surgically obstructed ET, injected with lipooligosaccharide from NTHi. The temporal bones were analyzed by light and electron microscopy. The goblet cell population of the ME mucosa showed an increase initially, but later decreased.

Mattsson et al[117] carried out studies to determine whether an increased production of free radicals is involved in the causation of myringosclerosis. Sclerotic lesions in the tympanic membrane were induced by keeping rats with perforated tympanic membranes in a hyperoxic atmosphere. The animals were treated daily with a solution containing either copper zinc–superoxide dismutase plus catalase and deferoxamine or copper sulfate plus iron chloride. The results showed that treatment with copper zinc–superoxide dismutase plus catalase and deferoxamine inhibited or reduced the development of myringosclerosis, whereas treatment with copper sulfate plus iron chloride produced no effect. The authors concluded that the formation of oxygen free radicals contributes significantly to the development of myringosclerosis.

The aim of a study by Russell and Giles[118] was to create a suitable mechanical rat model that would produce a sterile and long-lasting effusion. To achieve their objective, the researchers blocked the ETs of rats in the mid-portion along the skull base. Their results suggest that this model is reliable for creating a persistent and long-lasting effusion.

The aim of a study by Ikarashi et al[119] was to determine whether gas exchange occurs in the ME air cell system independent of the ET. The authors blocked the ME clefts of piglets and investigated subsequent changes in the air cell system. Their results suggested that the normal ME air cell system has the capacity to perform gas exchange independently of the ET, with gas exchange occuring through the submucosal capillary network.

Von Unge et al[120] studied the changes in tympanic membrane stiffness in response to experimentally induced cholesteatoma by obstruction of the ear canal in gerbils. Their results suggest that the stiffness changes correlated with an increased overall tympanic membrane thickness and increased thickness of the lamina propria. The authors concluded that the stiffness changes may play an important role in the pathophysiology of cholesteatoma.

The purpose of a study by Goycoolea et al[121] was to evaluate factors involved in the development of OM that could play a role in the pathogenesis of acquired cholesteatoma. The investigators evaluated the temporal bones of cats and chinchillas with experimentally induced OM, as well as those from humans with OM. The authors conclude that in order for transmigration of squamous epithelium to occur, a trigger (inflammatory process) and a bridge (granulation tissue and organized effusion) are needed.

The goals of a study by Meek et al[122] were to develop a mouse model for virally induced OM and to study the immune response to the infection. Intranasal inoculation of mice by reovirus was used to induce OM. Immunohistochemical evidence for the presence of reovirus in the nasopharynx, ET, and ME, as well as the amount of infiltrating B cells and T cells in these sites, was evaluated. The authors could detect reovirus antigen in the ME mucosa of infected animals, and histologic evidence for OM was found in half of all infected animals. A significant increase in B cells in the nasopharynx and ETs was also seen after the infection. The number of infiltrating T cells, however, did not vary significantly from that in the control animals.

AQUAPORINS

Chun et al[34] studied the expression of the aquaporins in rat ME and ET mucosa and in ME and ET cell lines by reverse transcriptase–polymerase chain reaction and immunolabeling. Transcripts of all aquaporins, except for AQP 2 and AQP 6, were expressed in the cell lines and tissues.

SHORT-TERM RECOMMENDATIONS

1. Study normal autonomic innervation involved in regulation of the vasculature and secretion of the tubotympanum.
2. Characterize the lymphatics of the tubotympa-

num.

3. Develop a more detailed 3-D model for education and research in order to better understand the physiology and pathophysiology of the tubotympanum.

4. Characterize the cellular and molecular interactions between the host and pathogens.

5. Characterize secretory function of the tubotympanum in normal and pathological conditions.

6. Determine the usefulness of the cell models, including primary cultured cells and cell lines.

7. Establish animal models, including transgenic or gene knockout, to study normal biology and pathogenesis of OM.

8. Develop improved animal models for chronic mucoid OM.

9. Develop an animal model for genetic basis of host susceptibility to OM.

10. Develop a sensitive method to determine pathogens by means of archival human temporal bone sections.

11. Study the cellular and molecular bases for OM sequelae such as atelectasis, granulation, tympanosclerosis, and cholesteatoma.

12. Establish normal and pathological bone modeling and remodeling processes.

13. Characterize the cellular mechanisms involved in inner ear damage due to OM.

LONG-TERM GOALS

1. Establish the cellular and molecular mechanisms involved in the development of the tubotympanum.

2. Understand the cellular and molecular mechanisms involved in the pathogenesis of OM.

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5. MOLECULAR BIOLOGY AND BIOCHEMISTRY

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INTRODUCTION

Otitis media (OM) is a complex, multifactorial disease entity. The biology of OM is characterized by multiple interactions between the microorganisms that are involved in infections of the middle ear (ME) and the protective responses of the host. Inflammation associated with such protection appears to play a significant role in the etiology of OM, particularly chronic OM. Studies of the biochemistry of OM have therefore focused largely on the inflammatory biology of the ME. However, the behaviors of both pathogen and host involve a variety of other biochemical pathways to contribute to both pathogenesis and recovery. Both pathogen and host responses in the ME involve the expression of specific subsets of genes, and molecular biological techniques have also been applied to the study of OM. This powerful methodology has been used to identify pathogens, to clone bacterial genes as recombinant vaccine candidates, to evaluate the expression of host genes involved in the inflammatory and proliferative responses in the ME, and to induce gene expression in the ME. This section reviews recent advances in both biochemical and molecular studies of OM, and identifies goals for future research.

RECENT CONTRIBUTIONS TO BIOCHEMISTRY

The ME inflammatory response is typified by the accumulation of cellular and chemical mediators in ME effusions (MEEs). Information regarding the biochemistry of inflammatory mediators has advanced significantly in recent years. In particular, the number of identified cytokines continues to increase, while our understanding of the role played by cytokines in the initiation and regulation of inflammatory and immunologic responses has expanded dramatically. These and other developments have fueled significant advances in our understanding of the biochemistry of OM. However, specific biochemical and immunochemical factors that may be responsible for the severity or chronicity of OM have not yet been identified. Identification of factors involved in chronicity appears to be an essential step in the treatment and ultimate prevention of chronic OM.

INFLAMMATORY MEDIATORS

The composition of MEE reflects the inflammatory events taking place in the ME cavity. Inflammatory mediators released in the ME cavity appear to play an important role in the pathogenesis of OM and can cause functional, as well as morphological, changes in the ME.

Cytokines. Cytokines are a group of immunomodulatory glycoproteins that control inflammatory and immunologic reactions in OM. Cytokines are produced by a variety of cells, including epithelial cells, polymorphonuclear leukocytes (PMNs), monocytes, and lymphocytes. The presence of several cytokines (interleukin [IL]–1β, IL-2, IL-6, IL-8, tumor necrosis factor α [TNF-α], interferon-γ [IFN-γ], and transforming growth factor β [TGF-β]) in MEE of patients with OM with effusion (OME) has been reported in the past.

The proinflammatory agents endotoxin, TNF-α, IL-1β, and intercellular adhesion molecule–1 (ICAM-1) were all detected in MEEs in more than half of cases of chronic OME (COME).[1] It was observed that endotoxin, TNF-α, and IL-1β, but not ICAM-1, correlated positively. It was also found that more TNF-α, IL-1β, and endotoxin were found in culture-positive than in culture-negative MEEs.

A hypothesis that the chronic inflammation seen in COME is the result of an imbalance in the ratio of proinflammatory cytokines and inhibitors was proposed by Maxwell et al.[2] They reported the presence of TNF-α and TNF soluble receptor (an inhibitor) in 83% and 91% of effusions, respectively, and a statistically significant correlation was observed between these parameters. The TNF-α levels were significantly lower for children with multiple tube insertions. Higher levels of TNF-α were noted in those children in whom episodes of OM developed after tube placement. In an animal model, TNF binding protein was reported to attenuate vascular extravasation into the ME.[3]

The IL-8 levels in MEE were significantly higher in children with acute OM (AOM) than in children with OME. The IL-8 levels in MEE with bacteria were higher than in those without bacteria.[4] In the past, the level of IL-8 in MEEs was reported to be correlated with levels of IL-1β and TNF-α. In recent studies, the concentration of IL-8, which displays potent chemotactic activity toward neutrophils, was reported to be positively correlated with the total number and percentage of neutrophils in the effusion. The IL-8 concentration in MEE was used to predict the total number and proportion of neutrophils.[5] It was also concluded that the total number of neutrophils did correlate with the type of effusion. The levels of leukotriene (LT)–B4 and IL-8 were reported to be higher in MEEs from patients with AOM associated with bacterial and viral infection than in MEEs containing no pathogens. Antibiotic treatment failure was associated with LTB4 levels, and within 1 month, was associated with high IL-8 levels.

It appears that PMN-related inflammatory substances play an important role in delaying recovery or causing recurrence of AOM. Effective treatment of AOM may require antibiotics, as well as pharmacological agents that modulate PMN functions.[6]

Serum levels of IL-6, an acute-phase cytokine, may be a useful marker to differentiate between bacterial and nonbacterial AOM. Bacterial AOM showed higher levels of IL-6 than nonbacterial AOM. Serum IL-6 levels in pneumococcal AOM were significantly higher than the levels associated with other bacterial AOM.[7]

Interferon is detected in leukocyte cultures of MEE when stimulated by adenovirus, rhinovirus, coronavirus, respiratory syncytial virus, or influenza A virus. Interferon levels in MEEs were higher in OME with no infectious background than in other groups (OME with a history of recurrent AOM or a history of recurrent upper respiratory tract infection without OME).[8]

In experimental OM caused by inoculating *Streptococcus pneumoniae* into rats, up-regulation of messenger RNA (mRNA) for inflammatory cytokines (TNF-α, IL-6, IL-10) in ME mucosa was observed.[9] Inoculation of nontypeable *Haemophilus influenzae* similarly resulted in up-regulation of mRNA encoding several inflammatory cytokines.[10] Increases in endothelial nitric oxide synthase (NOS) and inducible NOS have also been observed in the ME during experimental OM.[9,11]

Intratympanic inoculation of recombinant IL-1β produced MEE, and cytologic and histologic findings of ME mucosa in mice were similar to those in endotoxin-induced OME.[12] It was also reported that the incidence of OME was lower in mice with anti–IL-1 receptor antibodies than in mice injected with endotoxin or recombinant IL-1β only.

In an in vivo rat model, production of growth-regulated gene product/cytokine-induced neutrophil chemoattractant (GRO/CINC) was reported[13] in ME lavage, and gene expression was seen in the ME mucosa after topical inoculation with lipopolysaccharide. GRO/CINC-1 is a rat chemokine with structural and functional homology to human IL-8.

In another animal study using mice, Johnson et al[14] reported the demonstration of thickening of the epithelial layer and infiltration of inflammatory cells in the subepithelial space by transtympanic injection of human IL-8. Bacteria-injected (heat-killed *S pneumoniae*) ears demonstrated findings similar to, but not as extensive as, those found in IL-8–injected ears.

Platelet activating factor (PAF) is one of the most potent biological lipid mediators in relation to inflammation. Furukawa et al[15] measured the PAF activity in human MEEs and reported higher levels of activity in mucoid effusions than in serous effusions. They suggested that PAF may play an important role as a mediator of the inflammatory responses in the pathogenesis of OME. Studies on local or systemic application of inflammatory mediators and tubotympanic pathophysiology have been reported. Intratympanic application of PAF induced mucociliary dysfunction and pathological changes in the tubotympanum.[16] Rhee et al[17] reported a significant increase in ME vascular permeability after injection of PAF into the bullae of chinchillas.

Maxwell et al[2] reported the presence of TNF-α and its inhibitor, TNF soluble receptor (TNFsolR), in MEE from OME patients. They hypothesized that the chronic inflammation seen in COME is the result of an imbalance in the ratio of proinflammatory cytokines and inhibitors. Ondrey et al[18] compared the presence of early-response cytokines in MEEs of adults and children. Relatively smaller amounts of IL-1β and TNF-α were observed in MEEs from adults than in those from children (12% versus 85%, and 8% versus 85%, respectively). Eighty-eight percent of the effusions from adults were serous in nature, and 67% of the patients had a known history of head and neck malignancy and irradiation of the temporal bone. They concluded that adult OM might represent a less severe and more chronic inflammatory state than pediatric OM.

Arachidonic Acid Metabolites. Progress has been made in the study of arachidonic acid metabolites (eicosanoids) and related inflammatory mediators such as PAF. Levels of PAF and leukotrienes (LTs) C4, D2, and E4 from children with OME were measured by radioimmunoassay.[15,19] Measurements of

LTB4 and IL-8 in 271 ME fluid samples from children with AOM showed an association between high LTB4 levels and bacteriologic failure, and between high IL-8 levels and recurrence of AOM within a month.[6] In chinchillas, significant basal levels of eicosanoid production were noted in normal ME mucosa, which were enhanced during infection. Prostaglandin E2 predominated.[20]

GROWTH FACTORS AND CELL SIGNALING

Transforming growth factor β is involved in the modulation of inflammatory cell migration and proliferation, extracellular matrix formation, and potent mononuclear leukocyte chemotactic factor. Cooter et al[21] reported the presence of TGF-β1 and TGF-β2 in human MEE. The mean levels of TGF-β1 were 100-fold greater than those of TGF-β2. The TGF-β1 levels were significantly elevated in the more chronic mucoid effusions as compared with serous effusions. Higher levels of both isoforms were present in MEEs in association with a history of previous tympanostomy tube placement, indicating the possible role they may play in the chronicity of the disease.

Angiogenic growth factors and their receptors were detected in the rat ME during experimental OM.[22] Delivery of the angiogenic growth factors (vascular endothelial growth factor [VEGF], and fibroblast growth factor–1) to the subepithelial space of the ME mucosa was achieved with osmotic minipumps,[23] factors bound to sepharose beads,[24] and cells genetically modified to deliver the factors.[24] In each case, production of new blood vessels was observed. Delivery of fibroblast growth factor–1 also resulted in proliferation of subepithelial connective tissue, but not the mucosal epithelium.[23,24] The results suggest that these factors mediate portions of the proliferative response in the ME mucosa during OM. Messenger RNA encoding markers for bone formation was also found to be up-regulated during experimental OM in the rat.[25] Finally, growth factors have been implicated in healing of tympanic membrane perforations,[26] and supplementation of growth factors appears to enhance healing.[27]

Recently, several new biochemical parameters were identified in MEEs and ME epithelial cells. Very high concentrations of soluble terminal complement complexes were observed in MEE, indicating a strong complement activation taking place in OME. Protectin (CD59) excretion into MEE was also detected, and this may suggest shedding of CD59 from the ME epithelial cells by complement activation and inflammation.[28] The nitric oxide (NO) metabolites nitrate and nitrite were reported to be present in MEE, indicating NO production in response to inflammatory stimuli in OME.[29] The presence of matrix metalloproteinase, which degrades the extracellular matrix in thick, mucoid effusions, has been reported.[30] Matrix metalloproteinases 2 and 9 were detected at higher levels in thick, mucoid effusions than in thin effusions.

Cultured ME epithelial cells of rats were reported to have the capacity to express iNOS with cytokine stimulation.[31] Interleukin-1β and TNF-α induce iNOS expression and may lead to NO production in ME epithelial cells. Nitric oxide may mediate mucin hypersecretion in chronic OME and contribute to the pathogenesis of OME.[31]

Free radicals have been implicated in the pathogenesis of OM. Superoxide dismutase was localized and measured in the ME mucosa of guinea pigs with and without infection.[32] The concentration of NO in MEE from humans and from experimentally induced serous OM was determined.[29] In a rat model, lipopolysaccharide (LPS)–induced OME was inhibited by treatment with *N*-nitro-L-arginine methyl ester (L-NAME), a competitive inhibitor of NO synthase, indicating that LPS-induced OME is mediated by NO.[33,34]

EFFECT OF TREATMENT ON BIOCHEMICAL COMPONENTS IN EXPERIMENTAL MEE

Treatment with inhibitors of LTs and/or PAF of LPS-induced OME in chinchillas demonstrated partial suppression of OME and marked reduction of inflammation and thickness of ME mucosa.[35] The LTB4 receptor antagonist SC-41930 was found to be effective in reducing inflammation in killed *Staphylococcus aureus*–induced OME in guinea pigs.[36] In cultured ME epithelial cells, dexamethasone was found to inhibit mucous glycoprotein secretion, suggesting the role of phospholipase A2 in mucous glycoprotein secretion.[37] Tanaka et al[38] reported that oxatomide alleviated the experimental OME induced by keyhole limpet hemocyanin. Erythromycin treatment of rat OME inhibited the accumulation of inflammatory cells such as neutrophils and macrophages in the MEE. Erythromycin suppressed the exudation of plasma protein and the production of LTB4, LTC4, and prostaglandin E2. Erythromycin also down-regulated L-selectin expression and inhibited up-regulation of Mac-1, a neutrophil adhesion molecule.[39]

CHOLESTEATOMA

Determining the presence of defects in the normal biology, biochemistry, and molecular biology of the major cellular constituents of a cholesteatoma is essential to the understanding of the pathogenesis of cholesteatoma. However, the molecular and cellular defects that result in the clinical hallmarks of acquired and congenital cholesteatomas — namely, invasion, migration, uncoordinated proliferation, altered differentiation, and aggressiveness — are not fully understood. Several factors have been reported to be involved in epidermis proliferation in cholesteatoma. Those factors include IL-1 receptors,[40] TGF-α,[41] IL-1α,[42] IL-1β,[43] and IL-8.[43] Several cytokines are also involved in bone resorption. They are IL-1α, IL-6,[44] IL-8,[44] and TNF-α.[45]

It appears that various cytokines play important roles both in proliferation of epidermis and also in the bone resorption observed in cholesteatoma. It is known that phospholipase C-γl plays a central role in signal transduction, cellular growth, differentiation, and proliferation. Park et al[46] reported the overexpression of phospholipase C-γl in cholesteatoma matrix. This may suggest a possible derangement of enhanced growth signal transduction in keratinocytes. Increased expression of a number of angiogenic growth factors has also been detected in cholesteatoma.[47]

Recently, the nuclear phosphoprotein p53 tumor suppressor gene was reported to be elevated 9- to 20-fold in cholesteatoma tissues as compared to the normal postauricular skin or tympanic membrane.[48] The data indicated a defect in cholesteatomas in the mechanisms that p53 engages (ie, cell cycle control or apoptosis or both).

SHORT-TERM GOALS

1. Continue to search for biochemical mediators responsible for mucosal hyperplasia and for chronicity or recurrence of OM.
2. Identify specific biochemical factors, either bacterial or host-derived, that contribute to mucin overproduction.
3. Explore the role, as opposed to the occurrence, of biochemical mediators in OM.
4. Explore the regulation of biochemical mediators during OM.

LONG-TERM GOALS

1. Continue to investigate the biochemistry of inflammation in OM, including inflammatory mediator production, receptors, and signal transduction.
2. Identify biochemical factors that contribute to destructive processes in OM, such as tympanic membrane degradation, cholesteatoma, bone remodeling, and adhesions.
3. Identify biochemical factors that contribute to tissue recovery following OM.
4. Utilize biochemical information to identify potential sites for therapeutic intervention aimed at preventing inflammation and destructive processes, and enhancing tissue recovery.

RECENT CONTRIBUTIONS TO MOLECULAR BIOLOGY

The past decade has seen molecular biology applied to problems in virtually every arena of biology and medicine. Molecular biology offers an expanding array of powerful methods that are increasingly used to study OM. The potential for existing and emerging molecular approaches to contribute to our understanding of OM and to aid the development of treatments is high. In this section, we review recent contributions to OM research using molecular approaches, and identify promising avenues for future studies.

Significant progress has been achieved over the past several years in applying molecular methods to the study of OM, in both its acute and chronic forms. These studies have taken the form of investigating 1) the pathogens present in OM and 2) the biology of host responses.

MICROBIOLOGY

Nontypeable Haemophilus influenzae. Smith-Vaughan et al[49] found that colonization of the nasopharynx occurs within weeks of birth in Aboriginal infants, persists through childhood, and contributes to OM development. This high carriage rate is due to concurrent and successive colonization with multiple NTHi strains. Using polymerase chain reaction ribotyping and randomly amplified polymorphic DNA (RAPD) to assess the diversity of P2 gene sequences, the authors found evidence to suggest that the P2 gene has been transferred between strains. The paradoxical observation, in this population, of prolonged colonization and often co-colonization of P2-identical strains and the concept of P2's being an important antigen for immunity is explained by Smith-Vaughan et al[49] as evidence for immune suppression or perhaps tolerance in these children.

St Geme et al[50] found that most NTHi strains express 1 or 2 antigenically related non-pilus adhesins known as the HMW1 and HMW2 proteins. Some strains lack these and instead express an adhesin known as Hia. Upon examination of 59 NTHi strains for the prevalence and distribution of the *hmw* and *hia* genes, 47 strains had sequences corresponding to *hmw* 1 or 2 genes and 9 had *hia*-homologous sequences; no strain had both. While these strains failed to demonstrate distinct genetic divisions, all strains containing the *hmw* sequences lacked the insertion element IS1016 that was nonetheless present in two thirds of *hmw*-deficient strains. Since this insertion sequence is classically associated with capsule expression in this organism, the authors conclude that *hmw*-deficient strains (thus, those expressing the Hia adhesin or neither of the 2 adhesins) probably evolved more recently from an encapsulated ancestor.

St Geme and Grass[51] found that the non-pilus adhesin proteins HMW1 and HMW2 are localized on the surface of the microorganism in a process that involves cleavage of a 441 amino acid (AA) N terminal fragment. Using cell fractionation and cryoimmunoelectron microscopy, the authors found that a periplasmic intermediate occurs suggesting the involvement of the Sec machinery. Ultimately, these proteins are partially released from the bacterial surface. An accessory protein known as HMW1B is localized in the outer membrane and serves to translocate HMW1 across the membrane. In the absence of HMW1B, HMW1 is unprocessed and is degraded in part in the periplasm. A better understanding of the way these proteins are processed and secreted may provide information relevant to the pathogenesis of *H influenzae*.

El-Adhami et al[52] describe a 26 kd outer membrane protein (OMP), OMP 26, isolated from a nontypeable isolate (289). Immunization with this isolated OMP had been shown to enhance clearance of NTHi from the lung in rats. Cloning, sequencing, and translation of the gene encoding OMP 26 revealed that this OMP is a hydrophilic polypeptide. The gene is relatively conserved among NTHi isolates. Two recombinant forms of OMP 26 were expressed and used to immunize rats: a full-length equivalent of a preprotein (28 kd) and a 26 kd recombinant protein. The former had greater efficacy, inducing a better cell-mediated immune response and higher serum antibody titers. The efficacy of the 28 kd recombinant protein was comparable to that of the native protein. The authors suggest that this OMP has significant potential as a vaccine candidate.

Dawid et al[53] found the existence of a regulation system in *H influenzae* used to control expression of HMW1 and HMW2 that is very similar to that used in eukaryotic cells. In some eukaryotic cells, long stretches of tandem repeats in the 5' untranslated region of a particular gene control expression of the gene product. In NTHi, the authors identified a series of 7 bp repeats in *hmw*1A and *hmw*2A promotors that control the expression of these 2 adhesins. These tandem repeats were found to lie between 2 distinct transcription initiation sites and exerted a suppressive effect such that increases in repeat number resulted in a decrease in production of HMW1 and HMW2, whereas a decrease in the number of repeats resulted in an increase in specific mRNA and protein production. Variations in the numbers of repeats occurred upon passage in vitro, as well as in animal models and in human infections. The authors conclude that this system of regulation is both unique among prokaryotes and also likely to play a role in virulence by increasing the organism's ability to be adaptive.

It is known that *H influenzae* requires heme for growth and utilizes both hemoglobin and hemoglobin-haptoglobin as heme sources. Ren et al[54] extended previous work in which they identified HgpA as a hemoglobin and hemoglobin-haptoglobin binding protein in NTHi. A mutation made in *hgp*A that did not result in an effect on binding or utilization of either heme source led to the identification of at least 1 other gene product involved in heme acquisition. A new locus *hgp*B was identified that encoded HgpB. A double *hgp*A *hgp*B mutant had reduced ability to utilize hemoglobin-haptoglobin; however, it was not compromised in its ability to use hemoglobin as a heme source. This latter observation lead to the isolation of a third, approximately 120 kd protein highly homologous to HgpA and HgpB. Because ability to acquire heme on the mucosal surface is a requirement for colonization of the host, these Hgp proteins are likely to be integral to pathogenesis of NTHi-induced disease.

Streptococcus pneumoniae. Weiser et al[55] found that *S pneumoniae* undergoes phase variation (resulting in changes in colonial morphology) that is linked with pathogenesis. Since transparent colony variants colonize the nasopharynx more efficiently in animal models and also express more LytA (*N*-acetylmuramoyl-L-alanine amidase), the authors examined whether an LytA-deficient variant differed in ability to colonize the infant rat nasopharynx. No differences were observed in ability to colonize the nasopharynx between the parent strain and the LytA-deficient mutant. The authors conclude that while LytA expression does undergo phase variation in association with colony morphology, factors other than LytA are responsible for intrastrain differences in ability to colonize.

Swiatlo et al[56] found that pneumococcal surface protein A (PspA) is a 65 kd protein with an α-helical N terminal 288 AA, followed by an 82 AA proline-rich region, then ten 20 AA repeats and a 17 AA C-terminus. The 3' half of this molecule is relatively conserved, whereas the 5' half is believed to be highly variable. Upon closer examination of 18 strains representing 12 capsule and 9 PspA serotypes, the authors found that the gene sequences encoding the leader peptide, proline-rich, and repeat regions are indeed highly conserved among *pspA* and *pspA*-like sequences; however, the α-helical region coding domain is highly variable among different strains.

Moraxella catarrhalis. Aebi et al[57] mapped the protective epitopes of copper ATPase (CopB) using a monoclonal antibody (Mab 10F3) directed against the CopB OMP that had previously been found to play a role in enhancing clearance of *Moraxella catarrhalis* from the lung in an animal model. This Mab was shown to exert complement-dependent bactericidal activity against *M catarrhalis*. By comparing the translated AA sequences of 2 Mab 10F3–reactive and 2 Mab 10F3–unreactive strains, the authors were able to localize the Mab 10F3 binding site to a 26 AA region. A 26-mer fusion protein was thus synthesized and used to immunize mice. The resulting antiserum reacted with CopB in Western blot analysis and also bound to the surface of both homologous and heterologous strains. Thus, this 26-mer region defines 1 protective epitope of the CopB OMP of *M catarrhalis*. Further investigation of this epitope appears warranted.

Du et al[58] cloned and sequenced the lactoferrin receptor genes from 2 strains of *M catarrhalis*. *Lfr* genes were found to be arranged in the following order: *lbpB* followed by *lbpA*, which is a gene arrangement commonly found in other lactoferrin and transferrin receptor operons. The deduced lactoferring-binding protein A (LbpA) sequences of the 2 isolates were found to be 99% identical, whereas the LbpB sequences were 92% identical. A third open reading frame located 1 nucleotide downstream of *lbpA* in both isolates was 98% identical. Recombinant LbpA and LbpB proteins were expressed and antisera to them raised to assay antigenic conservancy among a panel of *M catarrhalis* isolates. The authors found that antibody to recombinant LbpA was not bactericidal; however, that to recombinant LbpB was weakly so. Recombinant LbpA did not bind human lactoferrin; however, recombinant LbpB did. Therefore, the authors conclude that LbpB would be a better potential vaccine component. Bonnah et al[59] published a preliminary characterization of isogenic mutants deficient in LbpB, LbpA, and Ofr3 proteins, as described in Du et al.[58]

Luke et al[60] used an isogenic *M catarrhalis* mutant and a monoclonal antibody (11C6) to identify a protective epitope of OMP B1, a human transferrin binding protein. Thirty-one percent of the clinical isolates tested demonstrated the presence of an OMP B1 epitope recognized by Mab 11C6. The authors suggest that OMP B1 represents a potential vaccine antigen.

HOST RESPONSES

Mucin Gene Expression in ME and Up-Regulation of Mucin in OM. Mucins are high–molecular weight glycoproteins that constitute the major component of mucus in the ME, trachea, and digestive and reproductive tracts. They protect epithelia and trap particulates including bacteria for mucociliary clearance. In diseases such as OM, chronic bronchitis, and cystic fibrosis, mucin production is up-regulated, in turn contributing to the pathogenesis of diseases. In OME, overproduced mucin is believed to play an important role in causing conductive hearing loss, as well as defective mucociliary clearance and recurrent infection.

Progress has been made in the study of mucin gene expression in the ME and eustachian tube. Chun et al[61] examined human mucin gene expression and found that mucin *MUC2*, *MUC5AC*, and *MUC5B* genes are expressed in human ME epithelial cells. A similar result was also found in human primary cultures of ME epithelial cells by Moon et al.[62] In addition to mucin gene expression in ME, Sone et al[63] also reported the expression of glycoconjugates in human eustachian tubes.

More attention has been given to determining mucin expression in ME mucosa and effusions in OM. Hutton et al[64] isolated and purified high–molecular weight mucins from ME effusions of children with OME and provided direct evidence for expression of different mucin gene products. Consistent with this study, Jung et al[65] reported that human *MUC5AC*, *MUC5B*, *MUC7*, and *MUC8* are all expressed in ME mucosa of patients with chronic OM. In the study reported by Severn et al,[66] human *MUC1* and *MUC2*, in addition to *MUC5AC* and *MUC5B*, were found to be expressed in ME mucosa.

Efforts have also been made toward understanding the molecular mechanisms by which mucin overproduction is caused. Li et al[67] investigated the role of *H influenzae* in mucin up-regulation and found that *H influenzae* up-regulates mucin gene transcription via activation of a Rac-dependent MEKK-SEK-p38 mitogen activated protein kinase pathway, suggesting that *H influenzae* up-regulates mucin transcription via activation of an intracellular signaling pathway different from that of *Pseudomonas aeruginosa*.[68] In addition to the involvement of p38, Xu et al[69] reported that oxygen radical species are also involved in *H influenzae*–induced mucin up-regulation. The role of proinflammatory cytokines in mucin up-regulation was explored by Lin et al.[70] Their studies showed that TNF-α up-regulates mucin *MUC2* gene expression in the ME of rats. All of these studies suggested the complexity of regulatory mechanisms underlying mucin up-regulation in OM.

Growth Factors and Signal Transduction. Extracellular stimuli induce both physiological and pathological cellular responses via activation of signal transduction pathways extending from receptors on cell surface to the transcription factors in the nucleus. Therefore, investigating these signal transduction mechanisms will not only provide new insights into molecular mechanisms of physiological responses, development, and pathogenesis, but may also open up novel therapeutic targets for the treatment of ear diseases such as OM.

Progress has been made recently in the studies of signal transduction mechanisms involved in the pathogenesis of OM. Oehl et al[22] examined the expression of VEGFs and their receptors (VEGF-Rs), important receptor tyrosine kinases involved in normal and pathological angiogenesis, and found that VEGF, VEGF-C, VEGF-D, and VEGFR-2 (flk-1) were up-regulated in the mucosa of rat MEs inoculated with bacteria. DeFiore-Hyrmer et al[71] provided evidence to link protein tyrosine phosphorylation with NTHi adherence and actin nucleation. Sudhoff et al[47] documented the presence of angiogenic growth factors and receptors in cholesteatoma. Using immunocytochemistry, Huang et al[72] found epidermal growth factor, fibroblast growth factor, and platelet-derived growth factor in cholesteatoma keratinocytes, while Ishibashi et al[73] detected keratinocyte growth factor and receptor mRNA. Huang and colleagues[72,74] found evidence that ras, c-jun, and p53 may be involved in signaling from these receptors. Schilling et al[75] observed high levels of tenascin in proliferating cholesteatoma keratinocytes, suggesting a role for integrin signaling. In addition, the role of the signaling molecule phospholipase C-γ1 was explored by Park et al.[76] Their studies suggested a possible involvement of phospholipase C-γ1 in cholesteatoma pathogenesis.

Expression of interleukins has been documented by several investigators. In general, genes encoding proinflammatory cytokines are expressed very rapidly, within 6 hours of ME inoculation with bacteria, and decline by 24 hours.[9,10] Anti-inflammatory and immunoregulatory cytokines are expressed over a much longer time course, with some lasting for weeks.[25,77] Chun et al[78] also showed that MAP kinase ERK is involved in IL-1–induced down-regulation of surfactant protein B in the ME.

John and Nam[29] reported that the concentration of NO, an important intracellular second messenger, is higher in MEE. In experimental chronic OM, endothelial NOS mRNA is increased and iNOS gene expression is dramatically up-regulated.[11] In addition to the role of NO in the pathogenesis of OM, Juhn et al[78] provided evidence indicating that NO may be involved in the cochlear transduction process.

Nuclear factor κB (NF-κB), a transcription factor, has been shown to play an important role in inflammatory responses. One possible involvement of NF-κB is to regulate the transcription of proinflammatory cytokines. Ushikai et al[80] studied the role of NF-κB in IL-8 expression and showed that activation of NF-κB is involved in IL-8 gene expression in human adenoidal fibroblasts.

Molecular Studies of ME. Development of the ME was found to be sensitive to retinoic acid,[81,82] mediated in part by changes in expression of the anteriorizing gene *goosecoid*.[83] Additional regulatory genes found to be involved in ME development include the murine homolog of the *Drosophila* homeotic gene *spalt*[84] and paired class homeobox genes *Prx1* and *Prx2*.[85] Extending previous work, Takeuchi et al[86] found that T cells bearing Vgamma2/Vdelta2 receptors play a preferential role in OM.

Gene Transfer to ME Mucosa. Ryan et al[24] successfully implanted cells that had been stably transformed to secrete fibroblast growth factor–1 into the subepithelial compartment. Mondain et al[87] demonstrated that an adenovirus vector can be used to transduce cells of the adult guinea pig ME mucosa. Similarly, Dazert et al[88] demonstrated adenovirus transduction of mucosal cells in the neonatal rat. These studies demonstrate the feasibility of gene therapy in the ME cavity for the treatment of OM.

SHORT-TERM GOALS

1. Continue to identify genes (both bacterial and host) that contribute to OM pathogenesis.
2. Develop additional polymerase chain reaction–based and gene array–based methods for identification of disease pathogens in OM.
3. Develop improved mouse models of OM, to allow utilization of transgenic mice for the study of OM pathogenesis.
4. Apply emerging molecular techniques such as gene profiling, transfection, and transgenic methods to study OM.
5. Systematically determine expression of host genes involved in OM by quantitative methods such as gene array technology.
6. Investigate signal transduction mechanisms involved in the control of host gene expression in OM, including cell membrane receptors and cell signaling pathways.

LONG-TERM GOALS

1. Develop new vaccines for OM prevention by

means of recombinant techniques.

2. Characterize antibiotic resistance in bacterial pathogens responsible for OM at the molecular level.

3. Understand bacteria-host interactions at the molecular level.

4. Identify host genetic factors that contribute to OM susceptibility, pathogenesis, and chronicity.

5. Characterize mechanisms by which transcription of critical host genes, including secreted products such as mucin, inflammatory mediators, regulators of hyperplasia, and protective substances, are regulated during OM.

6. Determine the involvement of key signaling pathways identified from in vitro studies using in vivo animal models and knockout mouse approaches.

7. Develop therapeutic strategies to inhibit the signal transduction pathways involved in the pathogenesis of OM.

8. Develop gene-based therapies for OM, including the use of gene delivery techniques and modulation of host or bacterial gene expression.

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6. MICROBIOLOGY AND IMMUNOLOGY

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During the past 25 years, significant advances have been made in our understanding of the immunology and microbiology of otitis media (OM). The report of the Sixth Research Conference on Recent Advances in Otitis Media,[1] as well as an excellent and timely review called "Recent Developments in the Immunology of Otitis Media,"[2] detail the basic framework of the microbiology and the immune system and its role in OM, and these will not be repeated here. It is the intent of this Report to review — culminating with this Symposium — progress that has been made since the last Research Conference and to delineate specific research goals for the next 4 years. The report of the panel on vaccinations appears elsewhere in this publication.

MICROBIOLOGY

During the past 4 years, substantial progress has been made in addressing many of the goals outlined at the 1995 Conference. The discussion that follows will highlight several important research observations that have been made in each of these areas.

ELUCIDATION OF INCIDENCE OF VIRAL INFECTIONS IN OM AND MECHANISMS BY WHICH THEY ENHANCE OR PREDISPOSE TO BACTERIAL INFECTIONS OF MIDDLE EAR SPACE

The contributions of respiratory viruses to the pathogenesis and clinical development of OM have been further supported by the work of several research groups. Several excellent studies have documented the incidence of viral infections in acute OM (AOM) and OM with effusion (OME) by a variety of detection methods. Pitkaranta et al[3] reported the results of a study using the reverse transcriptase–polymerase chain reaction (RT-PCR) to determine the frequencies of human rhinoviruses (HRV), respiratory syncytial virus (RSV), and coronaviruses (HCV) in a large number of children with AOM. Viral RNA was detected in 75% and in 48% of middle ear fluid (MEF) samples and 62% of nasopharyngeal aspirate (NPA) samples at the time of diagnosis. Human rhinovirus RNA was detected in both MEF alone and in NPA alone in 11%. Respiratory syncytial virus was detected in both MEF and NPA in 13% in MEF alone in 5%, and in NPA alone in 10%. Coronavirus RNA was detected in both MEF and NPA in 5%, in MEF alone in 2%, and in NPA alone in 10%. Bacterial pathogens were detected in 62% of MEFs from 91 children. Viral RNA was detected in 57% of bacteria-negative and in 45% of bacteria-positive MEF samples. No important differences in the risk of treatment failure, relapse, or occurrence of late secretory OM were noted between children with virus-positive and virus-negative MEF aspirates. These findings highlight the importance of common respiratory viruses in predisposing young children to AOM and also in causing it.

In a subsequent study, the same authors examined the incidence of respiratory viruses in children with OME.[4] Middle ear effusion (MEE) samples collected at the time of tympanostomy tube placement from 100 children with OME were examined. Viral RNA was detected in 30 children. Human rhinovirus was detected by RT-PCR in 19 children with OME and by virus isolation in 5 children. Respiratory syncytial virus RNA was found in 8 children and HCV in 3 children with OME. No dual viral infections were found. Bacterial pathogens were isolated from 35 MEE samples and were associated with viral RNA in 11 cases, 9 of them with HRV. These results suggest that viral infections — particularly HRV infection, either alone or concurrent with bacteria — are present in a larger percentage of children with OME than was previously suspected. It remains to be determined how often the presence of viral RNA in MEE represents persistent RNA, ongoing viral replication, or recurrent infection.

Heikkinen et al[5] also examined the prevalence of respiratory viruses in the middle ear during AOM. These workers determined the prevalence of various respiratory viruses in the MEF in 456 children (ages, 2 months to 7 years) with AOM. At enrollment and after 2 to 5 days of antibiotic therapy, MEF specimens and nasal wash specimens were obtained for viral and bacterial cultures and the detection of viral antigens. The viral cause of the infections was also assessed by serologic studies of serum samples obtained during the acute illness and convalescence. A specific viral cause of the respiratory tract infection was identified in 186 of the 456 children (41%). Respiratory syncytial virus was the virus most commonly identified in MEF: it was detected in the MEF of 48 of the 65 children (74%) infected by this virus ($p < .04$ for the comparison with any other virus). Parainfluenza viruses (15 of 29 children or 52%) and influenza viruses (10 of 24 children or 42%) were detected in the MEF significantly more often than enteroviruses (3 of 27 children or 11%) or adenoviruses (1 of 23 children or 4%) ($p = .01$ for all comparisons). Thus, RSV was the principal virus invading the middle ear during AOM.

In an interesting study also pertaining to the contribution of RSV to the development of AOM, Andrade et al[6] investigated the prevalence and the etiology of AOM in children with bronchiolitis. A primary goal was to determine whether AOM in these children was due entirely or mainly to RSV, in which case routine antimicrobial treatment would not be appropriate. The study group consisted of children 2 to 24 months old with bronchiolitis. Forty-two children with bronchiolitis were enrolled. Sixty-two percent had AOM at entry or developed AOM within 10 days. An additional 24% had OME either at entry or eventually. Only 14% remained free of both AOM and OME throughout the 3-week observation period. All patients with AOM had 1 or more bacterial pathogens isolated from 1 or both middle ear aspirates. Of 33 middle ear aspirates, *Streptococcus pneumoniae* was isolated in 15, *Haemophilus influenzae* in 8, *Moraxella catarrhalis* in 8, and *Staphylococcus aureus* in 2. Thus, the authors concluded that when AOM developed in patients with bronchiolitis, bacteria were always present in the MEFs and antimicrobial therapy was indicated.

Several studies published since the 1995 Research Conference have focused on the mechanisms by which viral infection predisposes to bacterial infection of the middle ear space. Chonmaitree et al[7] examined the role of leukotriene (LT) B4 and interleukin (IL)–8 in acute bacterial and viral OM. Levels of LTB4, a potent inflammatory product of polymorphonuclear leukocytes (PMNs), and IL-8, a PMN chemotactic cytokine, were measured in 271 MEFs from 106 children with AOM. Forty-two percent of the patients had evidence of respiratory viral infection. At the time of diagnosis, levels of both LTB4 and IL-8 were higher in the MEFs of patients with AOM associated with bacterial or bacterial and viral infection than in those MEFs containing no pathogen ($p \leq .05$). Antibiotic treatment was not associated with a significant change in levels of LTB4 or IL-8 in the MEFs obtained 2 to 5 days into treatment, as compared to those obtained at diagnosis. Bacteriologic failure after 2 to 5 days of treatment was associated with high LTB4 levels in the initial MEFs ($p = .05$). Recurrence of AOM within 1 month was associated with high IL-8 levels in the initial MEF ($p = .04$). These findings suggest that LTB4 and IL-8 are produced during acute infection of the middle ear, and these PMN-related inflammatory chemokines may play an important role in delaying recovery from AOM or in its recurrence. Effective treatment of AOM may require eradication of bacteria by antibiotics, as well as pharmacological agents that modulate PMN functions.

In a related study, Garofalo et al[8] examined the molecular mechanisms responsible for the production of IL-8 by RSV-infected A549 type II pulmonary epithelial cells. Infection with sucrose-purified RSV produced a time-dependent increase in the transcriptional initiation rate of the IL-8 gene. Transient transfection of the human IL-8 promoter mutated in the binding site for nuclear factor κB (NF-κB) demonstrated that this sequence was essential for RSV-activated transcription. Gel mobility shift assays demonstrated RSV induction of sequence-specific binding complexes; these complexes were supershifted only by antibodies directed to the potent NF-κB transactivating subunit RelA. Both Western immunoblot and indirect immunofluorescence assays showed that cytoplasmic RelA in uninfected cells became localized to the nucleus after RSV infection. RelA activation requires replicating RSV, because neither conditioned medium nor ultraviolet-inactivated RSV is able to stimulate its translocation. The authors concluded that RelA undergoes changes in subcellular distribution in airway epithelial cells upon RSV infection. The ability of replicating RSV to activate RelA translocation may play an important role in activating IL-8 and other inflammatory gene products necessary for the airway mucosal inflammation seen in RSV disease.

One additional study examined the relationship between the P5 fimbriae–mediated attachment of nontypeable *H influenzae* (NTHi) and infection with RSV.[9] The authors investigated the role of NTHi P5 fimbriae in attachment to RSV-exposed A549 epithelial cells. Analysis by fluorescence flow cytometry showed that a live P5-fimbriated NTHi strain (NTHiF+) attached to a higher proportion of RSV-exposed A549 cells than to control cells (mean, 68% for RSV versus 29% for control; p = .008), while attachment of the P5 fimbriae–deficient isogenic mutant strain (NTHiF–) was significantly lower than in control cells and rose only slightly after RSV exposure (mean, 17% for RSV versus 10% for control, p = .229). These data suggest that RSV infection significantly enhances NTHi attachment to respiratory epithelial cells. P5 fimbria appears to be the critical appendage of NTHi that participates in this attachment. In clinical settings, blocking of the P5 fimbria–mediated attachment of NTHiF+ by passive or active immunity may reduce the morbidity due to NTHi during RSV infection.

The relationship between influenza virus infection and isolation of middle ear bacterial pathogens was examined by Wadowsky et al.[10] Twelve allergic and 15 nonallergic adult subjects were intranasally inoculated with influenza A/Kawasaki (H1N1) virus. *Streptococcus pneumoniae* was not isolated from any subject before the virus challenge, but was isolated in heavy density from 4 (15%) subjects on day 6 (p = .055). The isolation rates of other middle ear pathogens were not significantly different before virus challenge and during the acute and resolution phases (days 27 to 30) of the experimental infection for the entire subject group or either the allergic or nonallergic subgroup. Densities and isolation rates of commensal bacteria from the entire subject group were similar throughout the observational period. These results suggest that experimental influenza virus infection promoted *S pneumoniae* colonization of the oropharynx and may explain some of the increased risk for development of OM associated with influenza infection.

FURTHER INVESTIGATION OF MEANS BY WHICH BACTERIAL CELL PRODUCTS INDUCE INFLAMMATORY RESPONSE AND LEAD TO CHRONIC CHANGES ASSOCIATED WITH MIDDLE EAR INFECTIONS

Significant progress has been made since the 1995 Research Conference in this area. Studies have ranged from identification of specific microbial factors critical for initiation of the inflammatory response to characterization of host inflammatory molecules and growth factors whose production is induced by infection. A number of relevant studies are cited below.

Sato et al[11] investigated the contribution of autolysin and pneumolysin to the middle ear inflammation caused by *S pneumoniae*. To explore the role of these factors in middle ear inflammation caused by pneumococci, they studied penicillin-induced inflammatory acceleration by using 3 related pneumococcal strains: wild-type strain 3 (WT3), its pneumolysin-negative derivative (P–1), and its autolysin-negative derivative (A–1). Both middle ears of chinchillas were inoculated with 1 of the 3 pneumococcal strains. During the first 12 hours, all 3 strains grew in vivo at the same rate, and all 3 strains induced similar inflammatory cell responses in MEF. Procaine penicillin G was given at 12 hours to half of the animals in each group, and all treated chinchillas had sterile MEF at 24 hours. Penicillin significantly accelerated MEF inflammatory cell influx into WT3- and P–1–infected ears at 18 and 24 hours in comparison to the rate for penicillin-treated A–1–infected ears. The inflammatory cell influx was slightly, but not significantly, greater after treatment of WT3 infection than after treatment of P–1 infection. The IL-1β and IL-6, but not the IL-8, concentrations in MEF at 24 hours reflected the penicillin effect on MEF inflammatory cells; however, the differences between treatment groups were not significant. These results suggest that pneumococcal OM pathogenesis in the chinchilla model is triggered principally by the inflammatory effects of intact and lytic cell wall products in the middle ear with, at most, a modest additional pneumolysin effect.

A number of studies have examined the contribution of inflammatory mediators induced by infection to the pathogenesis and persistence of disease. The study by Chonmaitree et al[7] cited above examined the role of LTB4 and IL-8 in acute bacterial and viral OM. Other investigators have performed related studies.

Diven et al[12] determined the role of various inflammatory mediators in the chinchilla model of pneumococcal OM. These investigators infected chinchillas with *S pneumoniae* and randomly assigned them to 1 of 4 treatment groups receiving intramuscular ampicillin sodium (control) or intramuscular ampicillin plus receptor blockers of platelet activating factor (WEB 2086), of leukotriene (MK 571), or of thromboxane A2 (GR 32191B). All treatments were begun on day 2 after inoculation and continued for 10 days. Differences among groups in the number of ears with effusion or in effusion volume were not statistically significant. In comparison to the control group, mucosal thickness and the number of ears with histopathologic signs of inflammation were significantly less in the GR and WEB treatment groups, but not the MK group. Also, effusion concentrations of free fatty acids, protease, and hydrolytic enzymes were significantly less in those groups. These results suggest that the addition of a receptor blocker for either platelet activating factor and/or thromboxane to ampicillin in the treatment of AOM reduces mucosal inflammation and decreases the production of other inflammatory chemicals.

Sutbeyaz et al[13] reported results of another, similar study assessing the ability of an LTB4 receptor antagonist to ameliorate the inflammatory process in a guinea pig model of OM. SC-41930 has been shown to be a specific LTB4 receptor antagonist both in vitro and in vivo. In this study, the anti-inflammatory effects of SC-41930 were investigated in a guinea pig model of OM induced by middle ear inoculation of killed *S aureus*. Outcome of treatment was determined by measurement of myeloperoxidase activity in the samples of middle ear mucosa, evaluation of temporal bone histopathology, and presence of MEFs. Myeloperoxidase activity in the SC-41930–treated group was found to be significantly lower than that in the control group. Histopathology of temporal bones indicated decreased inflammation in the treated group as compared to the controls. In addition, MEFs were absent in 4 of 6 treated animals. These results demonstrate that SC-41930 can produce significant anti-inflammatory effects in this model of OM and again point toward the significant contribution of LTs to the inflammatory process in the middle ear.

Host cytokines and chemokines have also been the focus of several investigations of the middle ear inflammatory process over the last several years. Johnson et al[14] investigated the contribution of IL-8 to the process. IL-8 is thought to control leukocyte recruitment and activation during inflammatory reactions, and the authors previously demonstrated the presence of IL-8 in chronically inflamed human MEEs. They hypothesized that IL-8 is responsible for the leukocyte recruitment seen in OM. For these studies, ICR mice were used to test the hypothesis that IL-8 could directly induce inflammation in the middle ear. Murine middle ears received 8-mL transtympanic injections of human IL-8 (25 mg/mL) in saline solution, heat-killed *S pneumoniae* (1 × 10^8/mL), or normal saline solution. The temporal bones were removed at 1, 4, 8, 24, and 48 hours, decalcified, and processed for histologic examination. Noninjected murine temporal bones served as controls. The normal saline–injected ears demonstrated little or no change as compared to the noninjected ears. The IL-8–injected ears histologically demonstrated thickening of the epithelial layer and subepithelial space, with inflammatory cell infiltration in the subepithelial space peaking at 4 to 8 hours and resolving by 48 hours. The bacteria-injected ears demonstrated findings similar to, although not as extensive as, those found in IL-8–injected ears (ie, inflammatory reactions peaked at 8 to 24 hours and resolved by 72 hours). These results demonstrate that IL-8 is a potent inducer of middle ear inflammation and support the concept that IL-8 may be one of the key cytokines responsible for the leukocyte accumulation and activation seen in OM.

More detailed studies of the early dynamics of the cytokine response were reported by Sato et al[15] for the chinchilla pneumococcal otitis model. They characterized middle ear inflammatory responses in the chinchilla OM model after injecting a very small number of viable pneumococci into the middle ear, similar to the natural course of infection. The MEF concentrations of IL-1β, IL-6, IL-8, and TNF-α were measured by use of anti-human cytokine enzyme-linked immunosorbent assay reagents. IL-1β showed the earliest peak, at 6 hours after inoculation, whereas the IL-6, IL-8, and TNF-α concentrations were increased 72 hours after pneumococcal inoculation. The IL-6, IL-8, and TNF-α, but not IL-1β, concentrations correlated significantly with total inflammatory cell numbers in MEF, and all 4 cytokines correlated significantly with MEF neutrophil concentration. Several intercytokine correlations were significant. Cytokines, therefore, participate in the early middle ear inflammatory response to *S pneumoniae*.

Finally, Willett et al[16] performed studies of children with chronic OME to assess the contribution of cytokines to the inflammatory process in the human host. Sixty-five MEEs and paired sera from 41 children with chronic OME were assayed for endotoxin and for tumor necrosis factor α (TNF-α) and IL-1β in order to establish whether a correlation exists between the concentrations of endotoxin and of these cytokines. Forty percent of the effusions had detectable levels of endotoxin, with a mean (±SD) concentration of 2.9 ± 7.8 endotoxin units per milligram of total protein. The mean concentration of TNF-α was 1.24 ± 3.1 pg/mg total protein, and that of IL-1β was 18.79 ± 2.5 pg/mg total protein. A strong, statistically significant correlation existed between the concentrations of endotoxin and TNF-α ($r = .89$) and IL-1β ($r = .72$). The data indicate that endotoxin may contribute to the pathogenesis of chronic OME by stimulating the sustained production of TNF-α and IL-1β in the middle ear.

FURTHER IDENTIFICATION AND CHARACTERIZATION OF RELEVANT ADHERENCE FACTORS FOR EACH OF COMMON MIDDLE EAR PATHOGENS AND IDENTIFICATION OF CORRESPONDING CELL SURFACE RECEPTORS

Substantial progress has been made since the 1995 Symposium in terms of advances in our understanding of the molecular pathogenesis of bacterial OM. Much of the attention has been focused on identification of bacterial adherence factors, molecular characterization of such factors, and characterization of the interaction of bacteria with eukaryotic cells on the cellular and molecular levels. The following discussion will focus on each of the major bacterial pathogens.

Haemophilus influenzae. A number of studies have been published since the last Symposium characterizing the adherence factors of NTHi. Several groups have focused research efforts on the pili expressed by these organisms.

The pilus gene cluster in *H influenzae* is known to consist of several genes. Van Ham et al[17] attempted to define the contribution of the major and minor subunits to the adherence of these organisms. The contribution of the major (HifA) and putative minor (HifD and HifE) subunits of *H influenzae* fimbriae to fimbria-specific adherence was studied by use of mutants that were inactivated in distinct fimbrial genes. Both the major and minor subunits were required for adherence of *H influenzae* to oropharyngeal epithelial cells and human erythrocytes carrying the AnWj antigen. Cloning of defined *H influenzae* fimbrial genes in an *Escherichia coli* strain with type 1 fimbriae yielded recombinants expressing high amounts of HifA-containing *H influenzae* fimbriae, either with or without co-expression of both *H influenzae* minor subunits. Both clones exhibited the specific adherence properties of *H influenzae* fimbriae — a finding implying that the minor *H influenzae* subunits are dispensable for adherence and that the adhesive domain resides in the major subunit, HifA. The authors speculate that in *H influenzae* itself, the minor subunits probably affect adherence by raising the number of fimbriae above the minimal level required to establish adherence.

Subsequently published studies have characterized the pilus gene locus in greater detail. Geluk et al[18] documented the frequency with which the intact fimbrial gene cluster was found in NTHi and characterized the heterogeneity between strains within the gene cluster. The occurrence of fimbria gene clusters in nonencapsulated *H influenzae* strains from chronic bronchitis patients (n = 58), patients with AOM (n = 13), and healthy carriers (n = 12) was determined by DNA hybridization and polymerase chain reaction (PCR), based on sequences of fimbriate *H influenzae* type b. Although an average of 18% of all nonencapsulated strains had a fimbria gene cluster consisting of *hifA* to *hifE* inserted in the chromosome between *purE* and *pepN*, differences in the frequency of fimbria cluster–positive strains were observed, depending on the source of isolates. The protein sequence of 3 ganglioside GM1–specific HifA adhesins consisted of conserved regions intermingled with regions of sequence diversity. HifA appeared to be flanked by intergenic regions that varied between strains and contained both direct and inverted DNA repeats. The presence or absence and the composition of the fimbria gene cluster and other sequences between the flanking genes *purE* and *pepN* suggest that the fimbria gene cluster was originally contained on a mobile element.

Clemans et al[19] subsequently characterized the hifA pilin gene in detail. Earlier experiments had demonstrated that the major epitopes of *H influenzae* pili are highly conformational and immunologically heterogeneous; their subunit pilins are, however, immunologically homogeneous. To define the extent of structural variation in pilins, which polymerize to form pili, the pilin genes (*hifA*) of 26 type a to f strains and 16 nontypeable strains of *H influenzae* were amplified by PCR and subjected to restriction fragment length polymorphism (RFLP) analysis. Six different RFLP patterns were identified. Four further RFLP patterns were identified from published *hifA* sequences from 5 NTHi strains. Two patterns contained only nontypeable isolates, and another pattern contained predominantly *H influenzae* type f strains. All other patterns were displayed by a variety of capsular and noncapsular types. The immunologic reactivity of pili with antipilus antisera correlated with the groupings of strains based on *hifA* RFLP patterns. Those strains that show greater reactivity with antiserum directed against *H influenzae* type b strain M43 pili tend to fall into one RFLP pattern (pattern 3), whereas those strains that show equal or greater reactivity with antiserum directed against *H influenzae* type b strain Eagan pili tend to fall into a different RFLP pattern (pattern 1). Sequence analysis of representative HifA pilins from typeable and nontypeable *H influenzae* identified several highly conserved regions that play a role in bacterial pilus assembly and other regions with considerable amino acid heterogeneity. These regions of HifA amino acid sequence heterogeneity may explain the immunologic diversity seen in intact pili.

McCrea et al[20] used a variety of techniques to further characterize the *hifD* and *hifE* genes and their corresponding protein products. They examined the immunologic and structural diversity of the HifD and HifE pilus subunits among type b *H influenzae* (Hib) and NTHi strains. Western immunoblot analysis revealed that antibodies directed against the C terminus of HifD and HifE from Hib strain Eagan bound to HifD and HifE proteins, respectively, of all piliated Hib and NTHi strains tested. Whole-cell enzyme-linked immunosorbent assays showed that antibodies specific for native HifD or HifE of strain Eagan also bound to all piliated Hib strains, but did not bind to the piliated NTHi strains. Antibodies against HifE of strain Eagan inhibited the binding of Hib to human erythrocytes, but did not inhibit the binding of

NTHi strains. RFLP analysis was used to determine strain-to-strain structural differences within *hifD* and *hifE* genes, either by PCR or by nucleotide sequence analysis. DNA and derived amino acid sequence analyses of HifD and HifE confirmed the uniqueness of the RFLP types. The *hifD* and *hifE* genes of all type b strains showed identical restriction patterns. Analysis of *hifD* and *hifE* genes from the NTHi strains, however, revealed 7 unique RFLP patterns, suggesting that these genes encode proteins with diverse primary structures. These results indicate that HifD and HifE are immunologically and structurally similar among the Hib strains, but vary among the NTHi strains.

In addition to the bacterial pili that contribute to the ability of *H influenzae* to attach to host mucosal epithelial cells, several nonpilus bacterial adhesins have been identified and a number of studies characterizing various aspects of these molecules have also been published since the 1995 Symposium.

Barenkamp and and St Geme[21] identified a second family of high–molecular weight (HMW) adhesion proteins expressed by NTHi. This group had previously reported that 2 surface-exposed HMW proteins, HMW1 and HMW2, expressed by a prototypic strain of NTHi, mediate attachment to human epithelial cells. The HMW1/HMW2 proteins are members of a family of highly immunogenic proteins common to 70% to 75% of NTHi strains. However, NTHi strains that lack HMW1/HMW2-like proteins remain capable of efficient attachment to cultured human epithelial cells — a fact suggesting the existence of additional adhesion molecules. The investigators reasoned that characterization of HMW immunogenic proteins from an HMW1/HMW2-deficient strain might identify additional adhesion proteins. Using standard genomic screening techniques, they identified 1 clone that expressed a protein with an apparent molecular mass greater than 200 kd. Transformation of nonadherent *E coli* strain DH5α with plasmids containing the genetic locus encoding this protein gave rise to *E coli* transformants that adhered avidly to Chang conjunctival cells. Subcloning and mutagenesis studies localized the DNA conferring the adherence phenotype to a 4.8 kbp fragment, and nucleotide sequence analysis further localized the gene encoding the adhesion protein to a 3.3 kbp open reading frame predicted to encode a protein of 114 kd. The gene was designated *hia* for *H influenzae* adhesin. Southern analysis revealed an *hia* homologue in 13 of 15 HMW1/HMW2-deficient NTHi strains. In contrast, the *hia* gene was not present in any of 23 NTHi strains that expressed HMW1/HMW2-like proteins. Identification of this second family of HMW adhesion proteins suggests the possibility of developing vaccines based on a combination of HMW1/HMW2-like proteins and Hia-like proteins that would be protective against disease caused by most or all NTHi.

In a subsequent study, Krasan et al[22] examined adhesin expression in matched nasopharyngeal and middle ear isolates of NTHi from children with AOM. As noted earlier, the HMW1 and HMW2 proteins, Hia, and hemagglutinating pili have all been identified as important adherence factors in NTHi. To gain insight into the relative importance of these adhesins in nasopharyngeal colonization and localized respiratory tract disease, these investigators assessed their expression in matched nasopharyngeal and middle ear isolates of NTHi from 17 children with AOM. In all patients, including 11 with bilateral disease, the matched isolates were isogenic according to total protein profiles and genomic fingerprints. Of the nasopharyngeal isolates, 14 expressed only HMW1/HMW2-like proteins, 1 expressed only Hia, 1 expressed only pili, and 1 expressed both Hia and pili. Further analysis revealed concordance between nasopharyngeal isolates and the matched middle ear isolates for expression of the HMW1/HMW2-like proteins and Hia. In contrast, in the 2 children whose nasopharynges were colonized by piliated organisms, the corresponding middle ear isolates were nonpiliated and could not be enriched for piliation. Nevertheless, Southern analysis revealed that these 2 middle ear isolates contained all 5 *hif* genes required for pilus biogenesis and had no evidence of major genetic rearrangement. Thus, the vast majority of isolates of NTHi associated with AOM express HMW1/HMW2-like proteins, with expression present in both the nasopharynx and the middle ear. A smaller fraction of nasopharyngeal isolates express pili, while isogenic strains recovered from the middle ear are often refractory to enrichment for piliation.

Other groups have identified other molecules that may contribute to the ability of NTHi to adhere to cells, secretions, and intercellular matrix substances. Reddy et al[23] described binding interactions between major outer membrane proteins of NTHi and nasopharyngeal mucin. Mucins of the epithelial cell secretions modulate bacterial colonization. Their study was designed to understand the mechanism of mucin-bacterial interactions and, in particular, binding between NTHi and nasopharyngeal mucin(s). In an overlay assay, binding appeared to be mediated by the major outer membrane proteins P2 and P5 of the bacteria and sialic acid–containing oligosaccharides of mucin.

One group has focused its efforts on characterizing a lipid-binding adhesin of *H influenzae*.[24,25] Busse et al[24] characterized 13 clinical strains of *H influenzae*, including types b, d, and nontypeable, that recognized phosphatidylethanolamine (PE), gangliotetraosylceramide, gangliotriosylceramide (Gg3), sulfatoxygalactosylceramide (SGC), and, to a lesser extent, sulfatoxygalactosylglycerol (SGG) in their in vitro assay. A PE affinity matrix was used to purify an adhesin of approximately 46 kd from both type b and nontypeable *H influenzae*. This adhesin was a potent inhibitor of *H influenzae* Gg3 and PE binding in vitro, and polyclonal antibodies specific for this protein prevented the attachment of *H influenzae* Gg3 and PE and cultured HEp-2 epithelial cells in vitro. In their subsequent work, Hartmann and Lingwood[25] reported that brief heat shock treatment induces a long-lasting alteration in the glycolipid receptor-binding specificity and growth rate of *H influenzae*. After a 5-minute heat shock at 42°C, some strains of *H influenzae* showed a marked increase in binding to SGC and acquired the ability to bind to SGG in thin-layer chromatography overlays. Additionally, heat-shocked *H influenzae* cells showed an increased growth rate (twofold). After exposure of the organisms to brief heat shock, Western blotting of a surface extract of *H influenzae* with anti-bovine brain hsp-70 monoclonal antibody showed an increase in 2 protein bands at 82 and 60 kd. This antibody was a potent inhibitor of the binding of heat-shocked *H influenzae* to SGC and SGG, but had no effect on PE, Gg3, or Gg4 binding in vitro. This indicates that cell surface hsp-70–related heat shock proteins can mediate *H influenzae* attachment to sulfoglycolipids after heat shock.

Other groups have further characterized the specific molecules to which *H influenzae* may bind. Fakih et al[26] described the specific binding of *H influenzae* to minor gangliosides of human respiratory epithelial cells. Gangliosides are sialylated glycosphingolipids that serve as receptors for various bacteria. To investigate endogenous gangliosides of human respiratory epithelial cells as potential receptors for *H influenzae*, 3 strains, including NTHi 1479, isogenic fimbriated (f+), and nonfimbriated (f0) *H influenzae* type b 770235, were tritium-labeled and overlaid on 2-dimensional thin-layer chromatography plates containing either purified HEp-2 gangliosides or murine brain gangliosides. NTHi 1479 bound exclusively to 2 distinct minor ganglioside doublets with mobilities near that of GM1. NTHI 1479 also bound to a distinct ganglioside of human macrophages whose chromatographic mobilities closely resemble those of one of the NTHi-binding gangliosides of HEp-2 cells. *Hemophilus influenzae* type b 770235 f+ and f0 each bound to a different minor HEp-2 ganglioside doublet, with proportionately weaker affinity for a major ganglioside doublet. When 80% to 90% of sialic acid residues were enzymatically removed from HEp-2 gangliosides, NTHi 1479 binding was proportionately impaired, as compared with that in untreated controls. These findings support a role for specific gangliosides of specific cells as receptors for certain *H influenzae* strains.

Virkola et al[27] characterized the interaction of *H influenzae* with the mammalian extracellular matrix. Specifically, the adhesiveness of 2 unencapsulated nonfimbriated strains of *H influenzae*, 23459 and 23330, and the encapsulated fimbriated strain 770235 to extracellular matrix and to its isolated components was studied, as was the potential of *H influenzae* plasminogen receptors to enhance degradation of extracellular matrix and bacterial penetration through basement membrane. All strains exhibited efficient adhesiveness to reconstituted basement membrane and to extracellular matrix from cultured human endothelial cells. Strains 23459 and 23330 efficiently adhered to immobilized laminin, fibronectin, and various collagens. Strain 770235 adhered efficiently to fibronectin and type I and III collagens and with low efficiency to laminin. With all 3 strains, plasmin generated on *H influenzae* plasminogen receptors degraded laminin and fibronectin, as well as extracellular matrix from human endothelial cells. Plasmin bound on *H influenzae* cells also potentiated penetration of bacteria through a basement membrane preparation reconstituted on membrane filters. These results give evidence for a role of extracellular membrane adherence and plasminogen activation in the spread of *H influenzae* through tissue barriers.

Finally, Gorter et al[28] studied the stimulation of the adherence of *H influenzae* to human lung epithelial cells by antimicrobial neutrophil defensins. Patients with chronic obstructive pulmonary disease frequently have recurrent lower respiratory tract infections with nonencapsulated *H influenzae*. The infected mucosa of these patients is infiltrated with neutrophils, which on activation may release antimicrobial peptides, including defensins. Defensins isolated from neutrophils or from sputum samples of patients with chronic obstructive pulmonary disease did not kill *H influenzae* from these patients, but they did stimulate adherence of the bacteria to human bronchial epithelial cells in a time- and dose-dependent manner. Maximal stimulation was observed after 3 hours in the presence of ≥ 10 μg/mL defensins, resulting in 65 ± 36 colony-forming units per cell (61-fold increase). The enhanced adherence was not solely due to charge effects and was specifically blocked by $\alpha 1$-proteinase inhibitor. Because adherence is the first step in the onset of respiratory tract infections, these findings indicate that neutrophil defensins probably contribute to the pathogenesis of *H influenzae* infection in the lower respiratory tract.

Streptococcus pneumoniae. Since the 1995 Symposium, significant advances have also been made in characterizing the molecular details of the adherence of *S pneumoniae* to eukaryotic cells. Several of the more recent investigations are highlighted below.

Pneumococci have been demonstrated to vary colonial morphology at a relatively high frequency between so-called opaque and transparent variants. This morphological variation has been associated with differences in adherence ability, and the molecular details of this variation have been the focus of several investigations. Cundell et al[29] described results of investigations in which they characterized the relationship between colonial morphology and adherence of *S pneumoniae*. Phase variants in colonial opacity of pneumococci differ in the ability to colonize the nasopharynx of infant rats. To explain this observation at a cellular level, these investigators compared the ability of opacity variants to adhere to buccal epithelial cells, type II pneumocytes, or vascular endothelial cells and to the glycoconjugates that represent the cognate receptors at each of these sites. The transparent phenotype was associated with enhanced adherence to buccal cells (approximately 100%) and their receptors relative to that of the opaque variants. Only modest differences in adherence (45%) were demonstrated for resting lung and vascular cells. In contrast, adherence of transparent variants increased by 90% for lung cells stimulated with IL-1 and by 130% for endothelial cells stimulated with TNF. In contrast, cytokine stimulation did not influence the adherence of opaque pneumococci. This difference correlated with the unique ability of transparent variants to adhere to immobilized *N*-acetyl-D-glucosamine (GlcNAc) and to cells bearing transfected platelet activating factor receptors. These results suggest that the mechanism of enhanced colonization of the nasopharynx in vivo by transparent as compared with opaque phase variants involves a greater ability to adhere to both GlcNAc β1-3Gal on buccal epithelial cells and GlcNAc and platelet activating factor receptors on cytokine-activated, as opposed to resting, lung and endovascular cells. In a subsequent study,[30] these workers examined the contribution of bacterial permeases of *S pneumoniae* to the adherence phenotype. Although pneumococcal adhesins are yet to be fully characterized, exported proteins on the bacterial surface are potential candidates. To identify bacterial elements involved in this process, mutants of *S pneumoniae* with defects in exported proteins were screened for the inability to adhere to cells representative of 3 in vivo niches: agglutination of bovine erythrocytes, which reflect adherence to cells that reside in the nasopharynx; human type II pneumocytes (lung cells [LCs]), representing the alveolar site of infection; and human vascular endothelial cells (ECs), representing the endovascular site. All of the 30 mutants analyzed produced hemagglutination values comparable with those of the parent strain. Four independent mutants demonstrated a greater than 50% decrease in adherence to both LCs and ECs. Sequence analysis of the altered alleles from these strains showed that mutations had occurred in 2 previously identified loci, *plpA* and *ami*, which belong to the family of genes encoding protein-dependent peptide permeases. Mutations in the *ami* locus resulted in an inability to recognize the GalNAc β1-4Gal glycoconjugate receptor present on resting LCs and ECs, whereas mutations in *plpA* resulted in a failure to recognize a GalNAc β1-3Gal glycoconjugate receptor also present on resting cells. Mutations in neither allele affected recognition of GlcNAc receptors present on cytokine-activated LCs and ECs. These results suggest that peptide permeases modulate pneumococcal adherence to epithelial and endothelial cells, either by acting directly as adhesins or by modulating the expression of adhesins on the pneumococcal surface during the initial stages of colonization of the lung or the vascular endothelium.

One possible adhesin identified at the time of the 1995 Conference Report was the surface protein named PsaA. Further characterization of this protein and the gene that encodes it has been reported by Berry and Paton.[31] The gene *psaA* encodes the 37 kd putative pneumococcal surface adhesin. Although its complete nucleotide sequence has been determined, its contribution to the pathogenicity of *S pneumoniae* had not been assessed previously. In this study, the investigators used a PCR-amplified internal fragment of the *psaA* gene from *S pneumoniae* type 2 strain D39 cloned in pVA891 to direct the construction of D39 derivatives in which the *psaA* gene had been specifically interrupted by insertion-duplication mutagenesis. Two independent D39 *psaA* mutants [PsaA-(1) and PsaA-(2)] were significantly less virulent than either the wild-type D39 strain or a derivative of PsaA-(1) in which the *psaA* gene had been reconstituted by back-transformation with an intact copy of the cloned gene. The adherence of PsaA-(1) to A549 cells (type II pneumocytes) was only 9% of that for D39. This is the first functional evidence that PsaA is an adhesin. Sequence analysis of the *psaA* gene from D39 indicated significant deviation from the sequence previously published for the homolog from *S pneumoniae* R36A. The deduced amino acid sequences of mature PsaA from the 2 strains had only

81% homology, with the bulk of the variation occurring in the aminoterminal portion.

More recent work has focused on the contribution of the so-called choline-binding proteins (CBPs) to the adherence capabilities of *S pneumoniae*.[32] The surface of *S pneumoniae* has recently been shown to be decorated with a family of CBPs that are noncovalently bound to the phosphorylcholine of the teichoic acid. Two examples (PspA, a protective antigen, and LytA, the major autolysin) have been well characterized. Rosenow et al[32] identified additional CBPs and characterized a new CBP, CbpA, as an adhesin and a determinant of virulence. By means of choline immobilized on a solid matrix, a mixture of proteins from a *pspA*-deficient strain of pneumococcus was eluted in a choline-dependent fashion. Antisera to these proteins passively protected mice challenged in the peritoneum with a lethal dose of pneumococci. The predominant component of this mixture, CbpA, is a 75 kd surface-exposed protein that reacts with human convalescent antisera. The deduced sequence from the corresponding gene showed a chimeric architecture with a unique N terminal region and a C terminal domain consisting of 10 repeated choline-binding domains nearly identical to PspA. A *cbpA*-deficient mutant showed a >50% reduction in adherence to cytokine-activated human cells and failed to bind to immobilized sialic acid or lacto-*N*-neotetraose, known pneumococcal ligands on eukaryotic cells. Carriage of this mutant in an animal model of nasopharyngeal colonization was reduced 100-fold. There was no difference between the parent strain and this mutant in an intraperitoneal model of sepsis. These data for CbpA extend the important functions of the CBP family to bacterial adherence and possibly identify a pneumococcal vaccine candidate.

At the time of the 1995 Conference Report, information was beginning to accumulate concerning the specific cellular receptors recognized by *S pneumoniae*.[33,34] Additional studies have been performed since that time. Barthelson et al[35] examined the ability of oligosaccharides to inhibit binding of *S pneumoniae* to respiratory epithelial cells. These investigators measured binding of live *S pneumoniae* organisms to a cultured cell line derived from the lining of the conjunctiva and to primary monolayers of human bronchial epithelial cells in the presence and absence of oligosaccharide inhibitors. Both encapsulated and nonencapsulated strains of *S pneumoniae* grown to midlogarithmic phase in suspension culture adhered to cultured primary respiratory epithelial cells and the conjunctival cell line. Adherence of 9 clinically prevalent *S pneumoniae* capsular types studied was inhibited preferentially by sialylated oligosaccharides that terminate with the disaccharide NeuAc α2-3(or 6)Galβ1. Adherence of some strains also was weakly inhibited by oligosaccharides that terminate with lactosamine (Galβ1-4GlcNAcβ1). When sialylated oligosaccharides were covalently coupled to human serum albumin at a density of approximately 20 oligosaccharides per molecule of protein, the molar inhibitory potency of the oligosaccharide inhibitor was enhanced 500-fold. The abovementioned experiments reveal a previously unreported dependence on sialylated carbohydrate ligands for adherence of *S pneumoniae* to human upper airway epithelial cells.

Related in vivo studies were performed by Idanpaan-Heikkila et al[36] in which they described the ability of oligosaccharides to interfere with the establishment and progression of experimental pneumococcal pneumonia. Specifically, oligosaccharides that block the adherence of bacteria to epithelial cells in vitro — lacto-*N*-neotetraose and its α2-3- and α2-6-sialylated derivatives — were tested for their abilities to attenuate the course of pneumococcal pneumonia and to prevent colonization of the nasopharynx in animal models. Intratracheal administration of these agents concurrently with bacteria dramatically decreased the pneumococcal load in the lungs of rabbits and conferred protection from bacteremia. The oligosaccharides ameliorated pneumonia and bacteremia when given therapeutically 24 hours after infection was established. When administered intranasally, neoglycoconjugates of the active oligosaccharides prevented colonization of the nasopharynx of infant rats. In addition to in vitro antiadherence properties, lacto-*N*-neotetraose acted directly on cultured lung epithelial cell lines to induce changes such that pneumococcal adherence was prevented for prolonged periods. These activities encourage continued development of oligosaccharides as a class of potentially preventive and therapeutic agents for infectious diseases.

Moraxella catarrhalis. At the present time, only very limited information has been published concerning the ligands and receptors critical to the attachment of *M catarrhalis* to host cells. Recently published data suggest that the UspA1 and UspA2, 2 closely related HMW proteins of *M catarrhalis*, may have some role in adherence.[37] McMichael et al[37] reported that purified UspA1 was found to bind HEp-2 cells; and sera against UspA1, but not against UspA2, blocked binding of the O35E isolate to the HEp-2 cells. UspA1 also bound fibronectin and appears to have a role in bacterial attachment. Purified UspA2, however, did not bind fibronectin, but had an affinity for vitronectin. Further characterization of these proteins should clarify their true role in adherence of these organisms.[37]

A study of potential importance to the understanding of *M catarrhalis* adherence was published by Fitzgerald et al.[38] These investigators reported that *M catarrhalis* adheres to human erythrocytes by means of a proteinaceous, trypsin-sensitive, heat-modifiable hemagglutinin. A 200 kd protein was found to be associated with hemagglutinating isolates of *M catarrhalis*. This protein was present on all hemagglutinating isolates (n = 17), but was absent on the nonhemagglutinating isolates (n = 23) examined. This protein demonstrated heat-modifiable properties in sodium dodecyl sulfate and was degraded by trypsin. Immunoblot assays with polyclonal antiserum indicated that the 200 kd protein was associated exclusively with hemagglutinating isolates, and antibodies to this protein did not recognize epitopes on nonhemagglutinating isolates. This surface protein may be a hemagglutinin of *M catarrhalis* and could potentially have a role in attachment of these organisms to mucosal epithelial cells in the nasopharynx.

CHARACTERIZATION OF MICROBIAL FACTORS CRITICAL FOR VIRULENCE OF PATHOGENS IN MIDDLE EAR

The 1995 Research Conference Report cited advances that had been made along several fronts in identifying virulence factors critical to the ability of the common middle ear pathogens to cause disease. Knowledge in this area has progressed even further over the past 4 years, and several of these important studies are outlined below.

Haemophilus influenzae. The contributions of both proteins and lipooligosaccharide (LOS) molecules present on the surface of NTHi have been the focus of investigation over the past 4 years. Variation in the major outer membrane proteins (MOMPs) has been proposed as an important means by which these organisms evade the host defenses. Duim et al[39,40] have published several studies on this topic. This group had previously reported on the natural variation of the P2 and P5 proteins during the course of natural infection in patients with chronic bronchitis. In the later work (summarized here), they tested the hypothesis that appearance of antigenic variants is the consequence of antibody-dependent selection in a rabbit model. The investigators produced infection with 1 of 2 strains of NTHi: d1 or d3. Persistence of *H influenzae* d1 was achieved in subcutaneous tissue cages for up to 948 days. During persistence in the rabbits, changes in MOMP P2 of *H influenzae* occurred that were similar to those observed in isolates from chronic bronchitis patients. In rabbits vaccinated with strain d3 and in nonvaccinated rabbits, antigenic drift occurred later than it did in rabbits vaccinated with strain d1. High titers of antibodies against *H influenzae* were measured in tissue cage fluid and serum. Vaccination of the rabbits with *H influenzae* d1 or d3 resulted neither in eradication of *H influenzae* d1 nor in increased antibody titers in serum and tissue cage fluid. The sera of nonvaccinated rabbits during persistence had no strain d1–specific bactericidal activity in the presence of complement. Vaccination with *H influenzae* d1 induced serum bactericidal activity against strain d1 in the presence of complement. However, a variant of strain d1 appearing in the tissue cages was not killed by this serum bactericidal activity. The authors concluded that immunologic pressure leads to the selection of MOMP variants of *H influenzae* and that these variants escape the antibody-mediated, strain-specific bactericidal activity against *H influenzae*.

In subsequent studies, this group characterized the changes in the P2 protein at a molecular level that occur in response to such immunologic pressure.[40] The antigenic drift resulted from amino acid substitutions in potentially surface-exposed loops of MOMP P2. The 8 loops of MOMP P2 of *H influenzae* d1 were separately expressed as fusion proteins with glutathione *S*–transferase. Sera of rabbits persistently infected with *H influenzae* reacted with the loop 5 and loop 6 fusion proteins in immunoblotting and enzyme-linked immunosorbent assay (ELISA). Rabbit sera contained antibodies reacting with peptides derived from loop 5 and peptides containing amino acids of loop 6. In addition, MOMP P2 variant-specific reactions with the amino acids located at the tip of loop 6 were detected. The rabbit sera showed variant-specific, complement-dependent bactericidal activities, which were eliminated by affinity chromatography with fusion proteins of loop 6, but not of loop 5. Thus, the authors were able to tentatively localize the specific target of bactericidal antibodies. They concluded that during persistence of *H influenzae* in rabbits, variant-specific bactericidal antibodies are elicited to the variable tip of MOMP P2 loop 6.

Similar molecular variation of the P5 protein has been reported in response to immunologic pressure. Duim et al further characterized the molecular changes responsible for such variability in the P5 protein.[40] The nature of this variability was determined by DNA sequence analysis of the P5 gene from 5 different *H influenzae* strains and their 7 MOMP P5 variants that were isolated from patients with chronic infections of the lower respiratory tract. Analysis of the P5 sequence data from the different strains revealed 4 well-defined, heterogeneous regions. These regions of variable sequence appeared to correspond to the regions of the gene encoding the putative surface-exposed loops of MOMP P5. The

MOMP P5 variants with alterations in MOMP P5 were shown to result from DNA point mutations and codon deletions. In addition, in 3 variants derived sequentially from 1 *H influenzae* strain, a frame-shift mutation resulted in the formation of a stop codon in the region encoding the signal sequence of the MOMP P5 gene. Strikingly, all nucleotide substitutions in the MOMP P5 loop regions of variants were nonsynonymous — a finding suggesting that variants with altered amino acid compositions of the surface-exposed parts of MOMP P5 obtained a selective advantage during persistence of the infection by nonencapsulated *H influenzae* in chronic bronchitis patients.

Other investigators have focused on the importance of LOS in the pathogenesis of NTHi infection. DeMaria et al[41] examined the relative virulence of a parent NTHi strain and 2 different LOS mutants. Recently, 2 NTHi LOS-deficient mutants were created and described. Strain 2019-DK1, an *rfaD* gene mutant, expresses a truncated LOS consisting of only 3 deoxy-D-manno-octulosonic acid residues, a single heptose, and lipid A. Strain 2019-B29, an isogenic *htrB* mutant, possesses an altered oligosaccharide core and an altered lipid A. Each strain's ability to colonize the nasopharynx and to induce OM subsequent to transbullar inoculation was evaluated in the chinchilla model. Compared with the parent and each other, the mutants demonstrated marked differences in virulence regarding their relative abilities to induce OM and persist in the middle ear after transbullar inoculation. Strain B29 required a 3-log–greater dose to induce OM as compared to the parent strain and did not exhibit evidence of sustained multiplication, but persisted for the same duration as the parent. Conversely, strain DK1, even when inoculated at a dose 4 logs greater than the parent dose, was eliminated from the middle ear 72 hours after challenge. Thus, the presence of intact LOS molecules appears to be critical to the virulence of NTHi in this model.

Other recent work has focused on variation and changes in the NTHi LOS molecules at a molecular level that appear to influence the interaction of the organisms with the host immune system. Weiser et al[42] reported that the LOS molecules are decorated with phosphorylcholine. Choline, although not a nutritional requirement for *H influenzae*, is taken up from the growth medium and incorporated into its LOS. Incorporated choline appears to be in the form of phosphorylcholine (ChoP) on the basis of the reactivity with the monoclonal antibody with specificity for this structure, TEPC-15. Incorporation of [^{3}H]choline from the growth medium and expression of the TEPC-15 epitope undergo high-frequency phase variation, characteristic of other LOS structures in this species. The expression and phase variation of ChoP require a previously identified locus involved in LOS biosynthesis, *lic1*. The first gene in *lic1*, *licA*, contains a translational switch based on variation in the number of intragenic tandem repeats of the sequence 5'-CAAT-3'. The full-length LicA polypeptide resembles choline kinases of eukaryotes, suggesting that the pathway for choline incorporation into the *H influenzae* glycolipid has similarities to the pathway for choline incorporation in eukaryotic lipid synthesis. The display of ChoP, a hostlike structure, renders the organism more, rather than less, susceptible to the bactericidal activity of human serum. The increased serum sensitivity of variants with ChoP correlates with higher serum immunoglobulin (Ig) G titers to LOS containing this structure. ChoP appears to be a cell surface feature common to a number of pathogens of the human respiratory tract, including *S pneumoniae* and mycoplasmas. In the case of *H influenzae*, its primary contribution to pathogenesis *does not* appear to be antigenic variation to evade host humoral clearance mechanisms.

In subsequent work, this group further explored the relationship between variation in LOS and interaction of NTHi with the host immune system.[43] Phase variation in colony morphology has been associated with the pathogenesis of infection caused by *H influenzae*. This study shows that differences in colony opacity of NTHi strain H233 involve phase changes in the lipopolysaccharide and depend on the expression of *lic1* and *lic2*, which contain translational switches based on intragenic tandem repeats of 5'-CAAT-3'. Genetic analysis showed that opaque organisms have an out-of-frame number of repeats in both *lic1*, required for the expression of ChoP, and *lic2*, a putative galactosyl transferase that adds the terminal galactose on Galα1-4Gal. Defined variants in these loci were used to examine the contribution of individual LOS structures to resistance to serum bactericidal activity mediated by antibody and C-reactive protein. The addition of ChoP by *lic1* was the only factor in serum killing involving C-reactive protein and complement. The terminal galactose moiety, in contrast, conferred resistance to killing by naturally acquired antibody and complement present in human serum. As Galα1-4Gal is also found on human glycolipids, it appears that decoration of the cell surface with this hostlike antigen blocks antibody-mediated serum bactericidal activity. Genetic analysis of NTHi within the human respiratory tract demonstrated that Galα1-4Gal may not be expressed during carriage, but that it may be advantageous for the organism in inflammatory states such as pneumonia.

Streptococcus pneumoniae. Investigators in the pneumococcal field have also advanced the study of virulence mechanisms at the molecular level. Both the pneumolysin and the surface carbohydrates of the organism have been the focus of detailed studies. Rayner et al[44] examined the contribution of the pneumolysin to virulence in an in vitro system. They studied the interaction of a pneumolysin-sufficient type II *S pneumoniae* strain (PL+) and an otherwise identical pneumolysin-deficient derivative (PL–) with human respiratory mucosa in an organ culture with an air interface for up to 48 hours. Ciliary beat frequency was measured by a photometric technique, and adherence to and invasion of the epithelium were assessed by scanning and transmission electron microscopy. PL+ and PL– caused a progressive fall in ciliary beat frequency as compared with the control that became significant ($p = .01$) at 24 hours for PL+ and at 48 hours for PL–. At 24 hours, there was a significant increase in the percentage of the mucosa of the organ culture that was damaged for PL+ as compared to the control ($p = .01$) and PL– ($p = .02$). At 48 hours, there was a significant increase in mucosal damage for both PL+ ($p = .005$) and PL– ($p = .05$) as compared to the control. At 24 and 48 hours, PL+ and PL– adhered predominantly to mucus and damaged cells. PL+ infection alone caused separation of tight junctions between epithelial cells, and at 48 hours, PL+ cells were adherent to the separated edges of otherwise healthy nonciliated cells. PL+ and PL– both caused damage to the epithelial cell ultrastructure. Infection with *S pneumoniae* caused patchy damage to the respiratory mucosa and a lowered ciliary beat frequency. These changes were more severe and occurred earlier with the pneumolysin-sufficient variant, thus confirming the importance of the pneumolysin in the pathogenesis of infection.

Kim et al[45,46] focused on the importance of the surface carbohydrates to the virulence of these organisms. As pointed out earlier, *S pneumoniae* undergoes spontaneous phase variation between a transparent colony phenotype and an opaque colony phenotype, the latter being more virulent in a murine model of sepsis. Opaque pneumococci have previously been shown to express lower amounts of C polysaccharide (cell wall teichoic acid), and in that study,[46] the investigators demonstrated by immunoelectron microscopy that the opaque organisms have a higher content of capsular polysaccharide. They then examined the relationship between expression of these 2 cell surface carbohydrate structures and their relative contribution to the increased virulence of opaque variants. Comparison of genetically related strains showed that the differential content of capsular polysaccharide did not affect the amount of teichoic acid as measured by a capture ELISA. In contrast, when the teichoic acid structure was altered by replacing choline in the growth medium with structural analogs, the quantity of capsular polysaccharide as measured by a capture ELISA was decreased, demonstrating a linkage in the expression of the 2 surface carbohydrate structures. A standardized assay was used to assess the relative contribution of cell surface carbohydrates to opsonophagocytosis. The opaque variants required 1.2- to 30-fold more immune human serum to achieve 50% opsonophagocytic killing than did related transparent variants (types 6B and 9V). The opsonophagocytic titer was proportional to the quantity of capsular polysaccharide, rather than to the quantity of teichoic acid. The major factor in binding of the opsonin, C-reactive protein, was also the amount of capsular polysaccharide, rather than the teichoic acid ligand. Only for the transparent variant (type 6B), which bound more C-reactive protein, was there enhanced opsonophagocytic killing in the presence of this serum protein. Increased expression of capsular polysaccharide therefore appeared to be a major factor in the decreased opsonophagocytic killing of opaque pneumococci.

More recently, in an animal model of systemic infection following intraperitoneal inoculation of mice, the opaque phenotype was significantly more virulent than the transparent phenotype for each of 3 strains examined.[46] The opaque phenotype was associated with 1.2- to 5.6-fold greater amounts of capsular polysaccharide as compared with the transparent phenotype on a sandwich ELISA. A similar technique comparing the amount of total teichoic acid showed that the transparent phenotype had 2.1- to 3.8-fold more detectable teichoic acid. This difference was confirmed by comparing the incorporation of $[^{3}\text{H}]$choline into teichoic acid. Cell fractionation revealed that variation in the quantity of incorporated choline was due to differences in cell wall–associated teichoic acid. These results suggest that the pneumococcus phase varies between a virulent form with more capsular polysaccharide and less teichoic acid and an avirulent form with less capsular polysaccharide and more teichoic acid.

CONTINUED MONITORING OF PNEUMOCOCCAL SEROTYPES CAUSING AOM AND INVASIVE DISEASE

Several groups have examined the pneumococcal serotypes responsible for disease in various populations. Many of these reports are from the more developed countries. Zangwill et al[47] reported in 1996 on the epidemiology of invasive pneumococcal disease in southern California. These investigators serotyped 22% of 360 isolates recovered from patients with invasive disease who were under 10 years of age. Serotypes 14 (24%), 23F (17%), and 19F (13%) were the most common causes of disease. Eighty percent of the isolates were among the serotypes included in the 9-valent pneumococcal conjugate vaccine currently in development. Eighty-six percent of the isolates represented serotypes included in the 23-valent polysaccharide vaccine. Similar results with slightly different percentages for individual serotypes were reported by investigators at the Centers for Disease Control.[48] They observed that overall, the most common causes of disease were serotypes 14 (21%), 9V (10%), and 6B (10%). When the analysis was restricted to patients under 6 years of age, the most common serotypes causing invasive disease were 14 (32%), 6B (15%), and 19F (9%). A more recent study by Kaplan et al[49] examined the serotype distribution and antimicrobial susceptibilities of more than 1,000 pneumococcal isolates from children with invasive disease in the United States between 1993 and 1996. The predominant serotypes were similar to those cited above, with the most common serotypes being serotypes 14 (28%), 6 (19%), and 19 (14%). Serotypes 6, 9, 14, 19, and 23 accounted for more than 75% of the isolates, and more than 95% of the isolates were in the serotypes/serogroups contained in the current pneumococcal polysaccharide vaccine.

Two relatively recent studies have reported on the pneumococcal serotypes carried in the nasopharynx of young children.[50,51] Dagan et al[50] obtained nasopharyngeal cultures from children taking part in a pneumococcal vaccine trial. Before immunization, *S pneumoniae* was isolated from 44% of the 247 children, 12 to 18 months of age, taking part in the trial. Of the serotyped isolates, the most common were serotypes 23F (25%), 6A (20%), 19A (14%), and 6B (11%). Homoe et al[51] studied nasopharyngeal carriage of middle ear pathogens among children in Greenland. Among control children, serotypes 19F, 6B, and 3 were found most commonly. Among children with AOM, the same serotypes were observed, but serotype 19A accounted for 35% of the pneumococci isolated.

FURTHER DEFINITION OF MICROBIOLOGY OF AOM AND CHRONIC OM IN DEVELOPING COUNTRIES

Studies on the microbiology of AOM and chronic OM in the developing world are still relatively limited. However, a few excellent studies have been published since the 1995 Research Conference. The study by Homoe et al[51] cited above examined the microbiology of OM among children in Greenland. Nasopharyngeal and middle ear colonization with bacteria, viruses, *Mycoplasma pneumoniae*, and chlamydiae was investigated in a survey of 54 children with AOM and 201 age-matched control children without AOM in Greenland. In total, 98% of the children with AOM and 91% of those without AOM carried potentially pathogenic bacteria in the nasopharynx. Two or more potentially pathogenic species were carried by 78% of the children with AOM and 57% of those without AOM. NTHi was found in 92% and 77%, respectively, but only *S pneumoniae* was found significantly more often in the nasopharynx of children with AOM than in the controls ($p < .03$). The 2 species were found in 22 of 24 ear discharge specimens. Nine children (3 with AOM) had chlamydiae in the nasopharynx, and 7 of them reported rhinitis. Enteroviruses or rhinoviruses were detected in 23 nasopharyngeal specimens from 39 children with AOM, in 13 such specimens from 39 children without AOM ($p = .040$), and in 4 of 14 ear discharge specimens. The potentially pathogenic load in the nasopharynx was massive, suggesting an association with the high prevalence of OM among children in Greenland.

Smith-Vaughan et al[52,53] have studied OM among Australian Aboriginal infants. Their particular interest has been the contribution of NTHi to the disease burden and the use of molecular techniques to characterize the strains. They used ribotyping with the restriction enzyme *XbaI* to study the dynamics of carriage of NTHi in Aboriginal infants at risk for OM.[52] The carriage rates of NTHi in the infants in the community were very high; the median age for detection was 50 days, and colonization was virtually 100% by 120 days of age and persisted at a high level throughout the first year of life. Eighteen different ribotypes of NTHi were identified from 34 positive swabs taken from 3 infants over a period of 9 months. The same ribotypes were recovered for up to 3 months from consecutive swabs of individual infants, and 12 of 27 swabs (44.4%) yielded 2 ribotypes from 4 colonies typed. Statistical analysis suggested that most swabs would have been positive for 2 ribotypes if enough colonies had been typed, although the second most frequent ribotype was detected on average in only 13% of strains. The authors reason that early colonization and carriage of multiple ribotypes of NTHi may help explain the chronicity of carriage and thus the persistence of OM in Aboriginal infants.

In an effort to possibly explain the pervasive and persistent carriage, the authors performed additional molecular studies.[53] They sequenced loops 4, 5, and 6 of the NTHi P2 porin gene and characterized several strains with prolonged carriage times. Despite a wide diversity of P2 gene sequences, they found 4 examples of P2 gene identity for strains with different genetic backgrounds as characterized by PCR ribotyping and randomly amplified polymorphic DNA typing, and speculated that the P2 gene had been transferred between strains.

UNDERSTANDING HOST AND ENVIRONMENTAL FACTORS THAT AFFECT COLONIZATION AND MICROBIAL ECOLOGY IN NASOPHARYNX

Studies have been published since the previous Symposium that more clearly defined the ecology and epidemiology of the common middle ear pathogens present in the nasopharynx. Of particular interest are studies that suggest that capsular transformation can occur in vivo, resulting in penicillin-resistant pneumococci of altered capsular types.

Faden et al[54,55] expanded their series of studies examining the epidemiology of colonization with potential middle ear pathogens and the relationship of such colonization to subsequent development of OM. In a study published in 1995,[54] they followed 200 children from birth through 2 years of age with nasopharyngeal cultures to determine the normal colonization pattern of NTHi. Forty-four percent of the children were colonized on 1 or more occasions, and the acquisition rate was greatest in the first year. The monthly prevalence rate was 11%. Colonization with the initial strain persisted 1 to 5 months (median, 2 months). Children carried 1 predominant strain at a time, but became colonized with up to 7 different strains (mean, 2.2 strains). Children colonized with a single strain for 2 or more months produced a greater nasopharyngeal secretory IgA response to NTHi than did children colonized with different strains (log 2.35 ± 0.68 U versus 1.89 ± 0.25 U of P6 secretory IgA per nanogram per milliliter of total secretory IgA; $p < .01$). The authors suggested that the duration of colonization with a strain and acquisition of a new strain may be affected in part by the local production of specific secretory IgA.

In a subsequent study,[55] these authors reported on the relationship between nasopharyngeal colonization and the development of OM. A cohort of 306 infants was followed from birth through 12 months to determine the frequency and duration of colonization and the risk of AOM and OME. *Moraxella catarrhalis* was the most commonly isolated bacterium. Infants colonized at 3 months of age or younger were at increased risk of AOM and OME. Early colonization with *M catarrhalis* revealed the greatest risk (relative risk [RR] = 1.24), especially for OME (RR = 1.57). There was a strong relationship between the frequency of colonization and OM ($r = .37$, p .001) for each pathogen. Although *S pneumoniae*, NTHi, and *M catarrhalis* are part of the normal nasopharyngeal flora during infancy, an increased rate of colonization may identify a subpopulation of children that is at increased risk of OM.

A problem of particular concern from a public health standpoint in the United States and elsewhere has been the emergence of penicillin-resistant pneumococci as a major cause of OM among children. Interesting studies have been published over the past few years exploring the dynamics of the nasal colonization process. A very troubling finding has been the observation that capsular transformation of resistant strains may occur in vivo. Barnes et al[56] examined this issue in some detail. Surveillance for nasopharyngeal colonization with *S pneumoniae* was maintained in a research day-care center between 1985 and 1992. An outbreak of nasal carriage of a multidrug-resistant (MDR) serotype 23F organism occurred between May 1990 and December 1991 and involved 14 of 52 children. Electrophoresis of penicillin-binding proteins and pulsed-field gel electrophoresis of chromosomal DNA indicated that the MDR serotype 23F organism was closely related to a serotype 23F MDR clone that has been prevalent in Spain since the early 1980s. In June 1991, an MDR serotype 14 organism was isolated from a child who had previously carried the MDR serotype 23F strain. Pulsed-field gel electrophoresis and typing of penicillin-binding proteins revealed that the MDR serotype 14 organism was very similar to the circulating MDR serotype 23F strain — a finding suggesting serotype transformation. These data suggest that dissemination of MDR pneumococcal strains and spread of the MDR phenotype to additional serotypes may be facilitated in group day care. More detailed molecular studies along these same lines were reported by Coffey et al.[57]

Serotype 19F variants of the major Spanish MDR serotype 23F clone of *S pneumoniae* have been proposed to have arisen by recombinational exchanges at the capsular biosynthetic locus. Members of the Spanish MDR serotype 23F clone and the serotype 19F variants were confirmed to be essentially identical in overall genotype, as they were indistinguishable by repetitive extragenic palindromic sequence based–polymerase chain reaction and had identical sequences at 3 polymorphic housekeeping genes. Eight serotype 19F variants were studied, and all had large recombinational replacements at the capsular biosynthetic locus. In all cases, 1 of the recombinational crossover points appeared to be upstream of *dexB*, which flanks 1 end of the capsular locus, and in 6 of the variants, the other crossover point was downstream of *aliA*, which flanks the other end of the locus. In 2 strains, a recombinational crossover point between the introduced serotype 19F capsular region and that of the Spanish serotype 23F clone could be clearly identified: within *cpsN* in 1 strain and within *cpsM* in the other. The differences in the recombinational junctions and sequence polymorphisms within the introduced capsular genes suggest that the 8 serotype 19F variants emerged on at least 4 separate occasions. Changes in capsular type by recombination may therefore be relatively frequent in pneumococci and would have implications for the long-term efficacy of conjugate pneumococcal vaccines that protect against only a limited number of serotypes.

CONTINUED MONITORING OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF MAJOR MIDDLE EAR PATHOGENS ON WORLDWIDE BASIS

A number of studies published since the 1995 Research Conference have reported on the antimicrobial susceptibility patterns of the major middle ear pathogens. The major area of concern has continued to be the increasing incidence of *S pneumoniae* resistant to penicillin and cephalosporins. Several of the more comprehensive studies[47-49] are highlighted below.

As mentioned above, Zangwill et al[47] reported on population-based prospective surveillance of invasive pneumococcal disease in Southern California. From March 31, 1992, to April 1, 1995, 814 cases were identified, for an incidence of 12.5 per 100,000 persons per year. The incidences among persons ≤2, ≤5, and ≥65 years of age were 145, 72, and 32 per 100,000, respectively. More than 95% of the cases included bacteremia; the incidence of meningitis was 0.8 per 100,000. Among children ≤2 years of age, 79% of isolates were obtained in the outpatient setting, as compared to 16% of isolates among persons ≥15 years of age. Eighty percent of isolates were serotypes included in heptavalent pneumococcal conjugate vaccines currently being evaluated. Children ≤2 years of age were at highest risk of having an isolate resistant to penicillin. Among resistant isolates, high-level resistance increased from 4% to 21% over a 3-year period.

Results of a similar national surveillance program were reported by Butler et al.[48] As part of ongoing national surveillance, serotyping and antimicrobial susceptibility testing were done on all pneumococcal isolates recovered from normally sterile body sites of patients at 12 hospitals in 11 states during 1993-1994. Of 740 isolates, 14.1% were penicillin-nonsusceptible *S pneumoniae* (PNSP; minimum inhibitory concentration [MIC] ≥ 0.1 μg/mL), 3.2% were penicillin-resistant (MIC ≥ 2.0 μg/mL), and 25.5% were nonsusceptible to more than 1 antimicrobial agent. PNSP were more prevalent among children <6 years old (18.4%) than among persons >18 years old (11.7%) and more prevalent among whites (16.2%) than among blacks (12.1%). PNSP represented 15 serotypes, but 89% of PNSP were serotypes in the 23-valent pneumococcal vaccine. The proportion of isolates with reduced susceptibility and the number of serotypes of nonsusceptible strains are increasing in the United States.

More recent data from the United States were published by Kaplan et al.[49] The study period was from September 1993 through August 1996. The patients studied consisted of infants and children with culture-proven systemic pneumococcal infection cared for at 8 children's hospitals in the United States. There were 1,291 episodes of systemic pneumococcal infection identified in 1,255 children. An underlying illness was present in the children for 27% of the episodes. The proportion of isolates that were nonsusceptible to penicillin or ceftriaxone increased annually and nearly doubled throughout the 3-year period; for the last year, the percentages of isolates nonsusceptible to penicillin and ceftriaxone were 21% and 9.3%, respectively. There was no difference in mortality rate between patients with penicillin-susceptible isolates and those with nonsusceptible isolates. Thus, the percentage of pneumococcal isolates nonsusceptible to penicillin and ceftriaxone increased yearly among strains recovered from children with systemic infection.

INCREASED UNDERSTANDING OF CONTRIBUTIONS OF NONCULTURABLE ORGANISMS AND CELL FRAGMENTS TO PATHOGENESIS OF OM

Since the 1995 Research Conference, a number of very provocative reports have appeared concerning the contributions of nonculturable organisms to the pathogenesis of OM. These studies suggest that organisms may persist in MEF much longer than previously appreciated and that they may contribute to the persisting inflammation present in chronic MEEs.

Aul et al[58] determined the persistence of culturable bacteria versus DNA in the presence of an MEE in a chinchilla model of OM. Cohorts of animals were either infected with ampicillin-resistant NTHi strains or injected with a tripartite inoculum consisting of freeze-thawed *S pneumoniae*, pasteurized *M catarrhalis*, and DNA from NTHi. The NTHi-infected animals displayed culture positivity and PCR positivity through day 35. In the chinchillas infected with the low–copy number inocula of *S pneumoniae*, DNA was not detectable after day 1 from the co-inoculated pasteurized *M catarrhalis* bacteria or the purified NTHi DNA; however, amplifiable DNA from the live low–copy number bacteria persisted through day 21, even though they were not culture-positive past day 3. These results demonstrate that DNA, and DNA from intact but nonviable bacteria, *does not* persist in an amplifiable form for more than a day in the presence of an effusion; however, live bacteria, while not culturable, persist in a viable state for weeks.

This initial study in animals was followed up by 2 complementary studies on samples collected from human subjects.[44,59] In the 1995 study,[44] the group attempted to determine whether PCR can detect bacterial DNA in pediatric MEEs that are sterile by standard cultural methods. The PCR-based detection systems for *M catarrhalis*, NTHi, and *S pneumoniae* were designed and validated with a battery of DNAs obtained from cultured bacteria. Specimens from chronic MEEs were collected and comparatively analyzed by culture and PCR. A total of 97 MEEs were collected from pediatric outpatients at Children's Hospital of Pittsburgh during myringotomy and tube placement for chronic OME (duration > 3 months). Of the 97 specimens of OME, 28 (28.9%) tested positive by both culture and PCR for *M catarrhalis*, NTHi, or *S pneumoniae*. An additional 47 specimens (48%) were PCR-positive and culture-negative for these 3 bacterial species. Thus, 75 of the 97 specimens (77.3%) tested PCR-positive for 1 or more of the 3 test organisms. The minimum number of bacterial genomic equivalents present in the average culture-negative ear was estimated to be greater than 10^4 in dilutional experiments. The authors concluded that PCR-based assay systems can detect the presence of bacterial DNA in a significant percentage of culturally sterile MEEs. While this finding is not proof of an active bacterial infectious process, the large number of bacterial genomic equivalents present in the ears is suggestive of an active process.

A report published by the same group in 1998 appears to support this idea.[60] The purpose of this latter study was to determine whether bacterial messenger RNA (mRNA), as detected by an RT-PCR–based assay, is present in chronic pediatric MEEs that contain bacterial DNA but are sterile by standard cultural methods. Bacterial mRNAs have a half-life measured in seconds to minutes; therefore, detection of bacteria-specific mRNAs would be evidence that metabolically active organisms are present. A total of 93 effusions were collected from pediatric outpatients seen for myringotomy and tube placement for chronic (>3 months) OME (median age of children, 17 months). The percentage of positive test results for RT-PCR–based assays was compared with culture results for *H influenzae* and concordance between RT-PCR–based findings and PCR-based findings for bacterial nucleic acids. Eleven of the 93 specimens (11.8%) tested positive by culture, PCR, and RT-PCR for *H influenzae*. A total of 29 specimens (31.2%) were positive by PCR but negative by culture for *H influenzae*. All 29 specimens were positive by RT-PCR for *H influenzae*–specific mRNA. The authors concluded that the RT-PCR–based assay system could detect the presence of bacterial mRNA in a significant percentage of culturally sterile MEEs, and that it established the presence of viable, metabolically active, intact organisms in some culture-negative cases of OME.

SHORT-TERM GOALS

Many research goals identified in the 1995 Report have been addressed at some length during the past 4 years. However, further studies to increase our understanding are still needed. Areas for proposed future study represent logical extensions of some of the more recent investigations. Short-term research goals that should be pursued over the next few years include the following.

1. Further evaluation of the relevant adherence factors for each of the common middle ear pathogens and identification of their corresponding cell surface receptors. Although substantial progress has been made in this area, a number of questions remain. The cell surface or other molecular receptors for the major pathogens are only beginning to be defined (with the exception, perhaps, of the pneumococcal receptor), and information in this regard is urgently needed. Although progress has been made in characterizing the adhesins of NTHi, the precise nature of the pneumococcal adhesion molecules still needs to be further defined, and little is known about adhesins expressed by *M catarrhalis*. This information may be directly applicable to development of effective vaccines for prevention of disease.

2. Examination of those microbial factors critical for virulence of pathogens in the middle ear. As the present report indicates, substantial progress has been made already in this area. Additional knowledge is still needed. The availability of the entire genomic sequences of several of the major middle ear pathogens, as well as the development of powerful new genetic tools for investigation of pathogenesis, should make the next 4 years a particularly productive time.

3. Improvement of molecular methods to enable the assays to detect a broader spectrum of viruses in volume-limited MEFs, and determination of the clinical significance of cases of "low-inoculum" infection as diagnosed by sensitive molecular techniques.

4. Further elucidation of the mechanisms by which viruses interact with bacteria and result in suboptimal clinical outcomes after antimicrobial treatment of AOM.

5. Continued monitoring of antimicrobial susceptibility patterns of the major middle ear pathogens on a worldwide basis and additional studies of the microbiology of AOM and chronic OM in developing countries of the world. Information in this area

is critically needed as we attempt to apply current knowledge in antimicrobial treatment and vaccine development to the rest of the world.

6. Increased understanding of the factors responsible for the translocation of bacteria and viruses from their normal location in the nasopharynx to their position in the middle ear space in which disease ensues.

LONG-TERM GOALS

Many of the goals identified in the 1995 Research Conference are still applicable today. In a global sense, the critical long-term goals are to attain sufficient understanding of the factors important in microbial virulence, and of the mechanisms by which infection leads to middle ear damage. This information is needed in order to develop specific approaches to intervene in a logical manner to prevent disease.

IMMUNOLOGY

SOURCE AND TRAFFIC OF LYMPHOCYTES TO MIDDLE EAR MUCOSA IN OM

Although the normal middle ear mucosa appears to be devoid of any organized lymphoid follicles, the middle ear mucosal epithelium integrates with other sites of the common mucosal immune system in the expression of local immune responses in the middle ear during OM. Data summarized in the previous Research Conference indicated that tonsils and adenoids may function as inductive sites for the immune response and serve as analogs in the upper respiratory tract for Peyer's patches. In accordance with the short-term goals from the previous Report, the manner in which lymphocytes gain access to the middle ear cavity has been a focus of intense research during the past 4 years. Adenoidal tissue produces secretory IgA antibody, and this antibody serves as the most important specific immunologic defense mechanism against colonization of the upper respiratory tract by potential pathogens. Two recent investigations using the outer membrane protein P6 from NTHi as a marker have correlated adenoid function with an immune response to this specific antigen from an OM pathogen. The data suggest that failure of adenoid lymphocytes to recognize specific immunogens may contribute to the development of recurrent OM,[61] and that the IgA immune response in the adenoids is largely responsible for the clearance of NTHi from the nasopharynx.[62]

Additionally, B and T lymphocyte subpopulations in the adenoids of children with OM have been established, as have Th1/Th2 cytokine profiles in the nasopharyngeal lymphoid tissue of children with recurrent OM.[63,64]

Finally, it has recently been demonstrated that adenoids participate in the development of immunologic memory and, on the basis of an evaluation of T cell subsets, that a relationship exists between allergy and memory cells in adenoids from children with OME.[65] These data provide an impetus for further investigation of the role of allergy in OM. Although a number of reports to the Seventh International Symposium addressed issues of lymphocyte homing and recruitment to the adenoid and tonsil,[66,67] the immunochemical and immunohistochemical features of the local adherence and retention of B cells in the middle ear after extravasation are still unknown.

IMMUNOREGULATION IN MIDDLE EAR

Data presented at the Sixth Symposium were consistent with the concept of local stimulation of IgA production during a middle ear immune response, mediated in part by local immune regulation induced by various cytokines. If mucosal lymphocytes are not preferentially recruited into the middle ear, increases in IgA seen during chronic middle ear disease may reflect altered local immunoregulation mediated by cytokines. A number of studies germane to the issue of immunoregulation in the middle ear have, since the previous Symposium, added to the growing list of cytokines, adhesion molecules, and T cell subsets identified in the middle ear. Transforming growth factor β (TGF-β) expression in OME was described by Cooter et al.[68] Their data indicate that TGF-β1 and TGF-β2 levels are elevated differentially during OME, suggesting that these isoforms may have differing roles during the inflammatory process in the middle ear. It is noteworthy that TGF-β has been found to stimulate class-switching of IgM B lymphocytes to an IgA phenotype. The precise role of TGF-β in immunomodulation in the middle ear during OM has not been determined.

Significant progress has also been made in elucidating the role of various cytokines in up-regulating adhesion molecules on endothelial and epithelial surfaces. The in vitro regulation of neutrophil migration by β2 integrins has been examined in patients with OM.[69] The role of IL-8 in the recruitment of neutrophils to the middle ear has also been further evaluated. The concentration of this potent neutrophilic chemoattractant and inflammatory cytokine in MEE from children with OM was found to correlate with the total number and percentage of neutrophils in the MEE and further supports the hypothesis that IL-8 contributes to the recruitment of PMNs to the middle ear.

Adhesive interactions between leukocytes and endothelium are required for subsequent leukocyte extravasation into the middle ear. The kinetics of expression of vascular cell adhesion molecule–1 in the middle ear has been examined during experimental lipopolysaccaride-induced OM.[70] The therapeutic potential of anti–cellular adhesion molecule antibody to block neutrophil migration into the middle ear and, thus, inflammation has also been examined and found to be effective in reducing middle ear neutrophils in vitro. The administration of anti-CD11b and -CD18 antibodies may be clinically useful for treating OM.[69]

ANTIBODY RESPONSE AGAINST SPECIFIC BACTERIAL ANTIGENS

The most common OM pathogens are *S pneumoniae*, NTHi, and *M catarrhalis*. While specific protective antigens or vaccine candidates of each are covered in the Microbiology and Vaccine sections of this report, several antigens have been specifically used as markers for basic immunologic investigations of the middle ear immune response. Outer membrane protein P6 from NTHi, which was a main focus of the previous report, continues to be evaluated. Data from Kodama et al[61] suggest that outer membrane protein P6 is a target for the cellular immune response of the adenoid and that failure of adenoidal lymphocytes to recognize this highly conserved antigen may be one of the causes of recurrent OM. Similar data obtained by these authors using serum antibodies directed against outer membrane protein P6, CD outer membrane protein of *M catarrhalis*, and capsular polysaccharides of *S pneumoniae* further suggest that persistence and/or recurrence of OME may be due to an insufficient serum antibody response to middle ear pathogens in young children.[71]

IMMUNE PATHOGENESIS OF OM

A number of investigations continued to address the issue of immune response, including allergy, as contributing to either the cause or the outcome of OM. Mast cell activity was demonstrated in a majority of MEEs from patients with chronic effusion whose atopic status had been characterized.[72] The data demonstrated that the cells and cytokines essential to the production of a Th2 immune-mediated hypersensitivity reaction are present in the middle ears of patients with OME. These data, together with the description of a relationship between allergy and memory cells in adenoids cited in the above section on the source and traffic of lymphocytes to the middle ear mucosa in OM, suggest that the role of allergy in OM needs further investigation.

SHORT-TERM GOALS

1. Various cytokines such as IL-1, Il-8, TNF-α, and INF-γ, which may modulate the extravasation of lymphocytes and other inflammatory cells, need to be examined for their role in the chronic forms of OM.

2. Epithelial cell biology should be investigated, particularly with regard to the ability of these cells to produce cytokines, engage in phagocytosis, and participate in mucosal inflammation, repair, and angiogenesis.

3. The role of adenoids and tonsils as the inductive sites for mucosal immunity should be explored further and related to functional aspects of the immune system. The ramifications of tonsillectomy and adenoidectomy on the mucosal immunologic response relevant to OM should be examined.

4. Evaluation of the immunogenetics of OM should be begun, to determine whether there is a genetic predisposition to a particular pathogen, tropism to particular tissue receptors, or a genetic basis for responders versus nonresponders to certain vaccines.

5. Further exploration should be made of the relationship between atopy, immune system hyperactivity, environmental triggers, and chronic inflammation of the middle ear by using new technologies that now permit an examination of Th1/Th2-type immune responses, as well as genetic predisposition to this altered reactivity.

LONG-TERM GOALS

1. The impact of the present practices used to treat acute OM needs to be studied in order to learn how the treatments could be modified to make them optimal for a protective immunologic response after acute OM.

2. Studies must be performed to define the immunologic defects previously observed in otitis-prone children.

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7. VACCINE

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RATIONALE FOR PREVENTION OF OTITIS MEDIA

There is much interest in developing methods to prevent otitis media (OM). Otitis media is an enormous national health problem in terms of both human suffering and cost. Physicians treat children more often for OM with effusion (OME) than for any other disease, and OME is the second leading cause of surgery in children, after circumcision.[1] Costs due to OM are estimated at a minimum of $5 billion annually.[1] Approximately 50% of infants have at least 1 OM episode by age 6 months, 80% by 1 year, and 90% by 2 years.[2] For children in whom OM develops within the first 6 months of life, recurrent OM or chronic OME is more likely to develop.[3-5] Recurrent OM is painful; it is associated with delay in speech and language development in infants and children, and it is associated with learning problems as children reach school age.[6,7]

FEASIBILITY OF PREVENTING OTITIS MEDIA BY VACCINATION

Prevention of OM is complicated by the multifactorial nature of the disease. Predisposing factors include eustachian tube dysfunction,[8] early colonization by nontypeable *Haemophilus influenzae* (NTHi),[9] immune dysfunction,[10] adverse interactions between respiratory bacteria and viruses,[10] and others. In view of these several determinants, one must question whether it will be feasible to develop a single vaccine to prevent OM.

Three lines of evidence indicate that a protective immune response can be generated to middle ear (ME) bacterial pathogens. First, while the early experience with pneumococcal vaccines meant to prevent OM was disappointing because of lack of immunogenicity, it is clear that a good serum antibody response is followed by type-specific protection from ME infection.[11-14] Therefore, if it is possible to overcome the poor immunogenicity of complex polysaccharide antigens in infants, prevention of OM due to *Streptococcus pneumoniae* by vaccination is a realistic goal. Second, the prophylactic administration to otitis-prone children of hyperimmune human immune globulin containing antibodies to pneumococcal polysaccharides resulted in a significant decrease in the incidence of pneumococcal OM.[15] Third, bactericidal antibody to NTHi is associated with protection from infection, and a strain-specific protective immune response develops in children after OM.[16] These 3 lines of evidence suggest it will be feasible to develop bacterial vaccines to prevent OM. One might hypothesize that despite the multifactorial determinants of OM, antibody to a protective antigen "tips the balance" against the occurrence of OM. The challenge is to identify the appropriate antigens and present them to the human host in such a way as to generate a protective antibody response in the ME and/or nasopharynx.

Characteristics of Ideal Vaccine for Otitis Media. Effective vaccines for OM must meet 4 major criteria.

1. They must be the "right" antigens; ie, antibody generated to the antigens will prevent the disease.

2. They must be conserved among strains of the bacterial species.

3. They must be a good immunogens, such that protective antibodies will be elicited in the population at risk (eg, infants and toddlers), and these antibodies must persist for sufficient time to provide protection through the risk period.

4. They should not increase ME inflammation via immune complex formation with etiologic bacterial and viral antigens.

A bacterial target antigen should have surface epitopes for binding by protective antibody, and the surface epitopes should be common among strains. Bacteria use antigenic variation of surface determinants as an adaptive mechanism to permit reinfection of hosts by different strains despite prior experience with the organism.[17]

PNEUMOCOCCAL VACCINES

It has been clearly demonstrated that pneumococcal capsular polysaccharides elicit a protective ME immune response. While there are 90 different capsular serotypes — many closely related — there is conservation of capsular polysaccharide immunogenicity among strains within a serotype. Several studies have demonstrated that capsular polysaccharide antigens did not reliably induce an antibody response in infants, but protection against OM by serotypes against an immune response in children was substantial.[11-14,18] This observation indicates that if capsular polysaccharide antigens can be presented to infants in a form that will reliably induce an antibody response, protection against pneumococcal OM might be conferred.

This Panel reported in 1995 that progress was being made in developing immunogenic vaccines consisting of pneumococcal capsular polysaccharides conjugated to protein carriers.[19-24] Conjugation increases polysaccharide immunogenicity by the recruitment of T lymphocyte help. Conjugate vaccines to prevent *H influenzae* type b (Hib) infections have been highly successful. They induce high-titer protective antibodies in infants, and have resulted in a dramatic decrease in the incidence of invasive Hib infections. Carrier proteins used in Hib and pneumococcal conjugate vaccines include tetanus and diphtheria toxoids, meningococcal outer membrane protein complex, and a mutant diphtheria toxin CRM197.

Pneumococcal conjugate technology is challenged by the necessity of including multiple serotypes in a polyvalent vaccine candidate, and the possibility that "antigenic competition" among the serotypes might inhibit the antibody response to some serotypes.[25] Other important questions must be addressed. Will a single protein carrier be effective for all serotypes? Will the large protein load required lead to adverse reactions? Standardized antibody assays are essential in achieving the goal of demonstrating vaccine protection.[25]

The Panel also reported in 1995 that alternative pneumococcal antigens were being investigated as potential vaccine candidates. Included were pneumococcal surface protein A (PspA), pneumolysin, and pneumococcal neuraminidase.[26-30] Substantial progress has been reported in the past 4 years with respect to pneumococcal conjugate vaccine efficacy in preventing OM.

Like the polyribosylribitol phosphate (PRP) capsule of Hib, pneumococcal capsular polysaccharides are T cell–independent antigens, which are poorly immunogenic in infants. Based on the observations by Avery and Goebel[31] that the coupling of a saccharide to a carrier protein yields a highly antigenic conjugate, polysaccharide-protein conjugate vaccines have been developed, first to Hib by means of PRP, and more recently, to pneumococcal capsular polysaccharides. The development of pneumococcal conjugate vaccines was recently reviewed.[32,33] After experiments demonstrated efficacy of pneumococcal conjugate vaccine against experimental acute OM (AOM) in chinchillas, clinical trials proved that these vaccines were immunogenic in infants.[34,35]

Two recent studies demonstrated that a pneumococcal conjugate vaccine (PNCRM7, Wyeth-Lederle Vaccines) is highly protective against invasive pneumococcal disease among infants and toddlers, and somewhat protective against OM. In both studies, the vaccine was administered at 2, 4, 6, and 12 to 15 months of age.[36,37] Among 37,000 California children in the Kaiser-Permanente Health Plan, immunized infants were protected against invasive pneumococcal disease after 7 months of age, as compared to the meningococcal vaccine control group.[36] The vaccine recipients had 8.9% fewer office visits due to OM, had 7% fewer new episodes of AOM, were 20.3% less likely to require tympanostomy tubes, and were 22.8% less likely to have recurrent OM (5 episodes in 6 months, or 6 episodes in 12 months) during the period from the first dose of vaccine to the end of the 3-year trial. In the Finnish trial, in which 831 children received PNCRM7 and 831 received hepatitis B vaccine as a control, the total number of AOM episodes was reduced by 6% (95% confidence interval [CI], 4%-16%) between 7 and 24 months.[37] Because all ears with AOM were tapped in the Finnish study, information about the causative organism was available. The number of AOM episodes attributable to pneumococcus was reduced by 34% (95% CI, 21%-45%), and the number due to vaccine serotypes was 57% lower in the PNCRM7 group than in the control group (95% CI, 44%-67%).

Several epidemiological studies have demonstrated that onset of OM during the first 6 months of life is one of the strongest risk determinants for recurrent OM and chronic OME.[4,5] Thus, an infant pneumococcal vaccine strategy, to have optimum impact on OM, should prevent ME infection early in life. Neither the Kaiser-Permanente study nor the Finnish

CHARACTERISTICS OF POTENTIAL VACCINE ANTIGENS OF NONTYPEABLE *HAEMOPHILUS INFLUENZAE*

Surface Antigen	*Molecular Mass (kd)*	*Surface Conservation*	*Bactericidal Antibody*	*Animal Protection*	*Adherence Factor*
OMP P1 (a)	45	No	Yes	Yes	No
OMP P6 (PAL)	16	Yes	Yes	Yes	No
OMP PCP	15	Yes	Yes	No	No
OMP 26	26	Not known	Not known	Yes	No
Pili	23-27	No	No	Yes	Yes
P5 fimbrin	36	No	Yes	Yes	Yes
HtrA/H91A (Hin 47)	47	Yes	No	Yes	No
Transferrin receptor	75-85	No	No	Yes	No
D15	80	Yes	No	Yes	No
HMW1/HMW2	100-150	No	No	Yes	Yes
Hia	100 to >250	No	No	Not known	Yes
LOS	3-5	No	Yes	Yes	No

study reported data on the OM-protective effect in the first 6 months of life. However, the theoretical impact of this vaccine on OM in the first 6 months is likely to be very small, since antibody responses in infants were much greater after 2 and 3 doses than after the first dose of conjugate vaccine.[34] Thus, infants are likely to remain susceptible to pneumococcal OM until 5 to 6 months of age. Prenatal third-trimester maternal pneumococcal immunization is currently being investigated to address this window of infant susceptibility.

Intranasal immunization with PspA, together with a nontoxic A subunit mutant of cholera toxin (mCT) S61F, induced significant PspA-specific immunoglobulin (Ig)–A antibodies in saliva and nasal secretions.[38] Further, significant numbers of mice intranasally immunized with PspA plus mCT S61F were protected from lethal challenge with capsular serotype 3 *S pneumoniae*.

Substantial progress has been made in the past 4 years in standardization of the pneumococcal capsular polysaccharide enzyme-linked immunoassay (ELISA), and in development of a functional opsonophagocytic assay. A reference serum from pooled human sera (89SF) has been made available by the US Food and Drug Administration, and concentrations of several IgG anti-pneumococcal capsular polysaccharide antibodies in this reference serum have been reported.[39] This reference serum is now being used by all major pneumococcal reference laboratories internationally to control for interlaboratory variation.

The first pneumococcal conjugate vaccine has now been licensed in the United States, and new conjugate vaccines will probably be licensed primarily on the basis of immunogenicity data rather than clinical efficacy. Analytical methods must be developed, evaluated, and validated in order to accurately compare immunogenicity results within and between laboratories for different vaccines. At present, no analytical technique is uniformly accepted and used in vaccine evaluation studies to determine the acceptable level of agreement between a laboratory result and the assigned value of a given serum. Plikaytis et al[40] described the magnitude of agreement among 12 laboratories quantifying an identical series of 48 serum specimens from 24 individuals (quality control sera) using a consensus IgG ELISA developed for their study. After assigning antibody concentration values to the quality control sera, they evaluated 4 methods for comparison of a series of laboratory-determined values with the assigned concentrations. The percentage error between assigned values and laboratory-determined concentrations proved to be the most informative of the 4 methods. This statistic benefits from its ease of implementation and interpretation. While the study focused on a pneumococcal IgG ELISA, the methods described can be easily generalized to other biological assays.

NONTYPEABLE *HAEMOPHILUS INFLUENZAE* VACCINES

Six potential NTHi vaccine antigen candidates were identified in the Report of the Sixth Research Conference.[41] All were proteins and were located on the bacterial surface. Genes encoding these proteins had been cloned, and the nucleotide sequences had been determined.

During the past 4 years, substantial progress has been made in identification of surface antigens of NTHi that may hold promise as vaccine candidates for prevention of disease. The Table summarizes the characteristics of a number of these candidate *Haemophilus* vaccine antigens. The report that follows highlights recent work from a number of laboratories that contributed important new knowledge to the field.

Outer Membrane Protein P1. Since the previous

Symposium, the role of outer membrane protein (OMP) P1 as a potential immunogen against NTHi-induced OM has not been directly investigated. However, Chong et al[42] assessed the role of the type b homologue as a potential vaccinogen against Hib-induced disease. A series of 13 synthetic peptides covering approximately 90% of the Hib P1 protein were used to identify linear B and T cell epitopes. Mouse, guinea pig, and rabbit antisera directed against purified native P1 identified 6 immunodominant B cell epitopes. Three common human linear B cell epitopes were also identified, and 4 regions of the P1 protein contained murine T cell epitopes. Eight peptide-specific rabbit antisera recognized P1 protein of both type b and nontypeable isolates, demonstrating the presence of highly conserved epitopes among strains. However, none of the antipeptide sera had bactericidal activity, nor were they protective against Hib challenge in the infant rat model of bacteremia. The results strongly suggested that the protective epitope(s) recognized by highly protective anti-P1 antibodies were conformational and could not be generated with linear P1 peptides. The same investigators[43] later created several glycopeptide immunogens by coupling synthetic oligosaccharides of the Hib capsular polysaccharide PRP (sPRP) to synthetic peptides containing potent T helper cell determinants and B cell epitopes of Hib OMPs P1, P2, and P6. The ability to induce high anti-PRP and anti-OMP IgG titers in rabbit sera was affected by the relative spatial orientation of sPRP to the T cell epitopes. Fifteen randomly selected rabbit antisera raised against several of these glycoconjugates were protective against Hib challenge in the infant rat bacteremia model. The results suggest it should be possible to design a strategy using immunodominant B and T cell epitopes of *H influenzae* OMPs as antigens and/or carriers for T cell–independent antigens to enhance protective antibody responses and induce cell-mediated immunity against typeable or nontypeable induced disease.

Outer Membrane Protein P6. Kyd et al[44] investigated the immunogenicity of P6 in a rat respiratory model. Rats were immunized with P6 purified from 2 NTHi isolates, a capsule-deficient strain and a Hib isolate, to assess their relative abilities to induce clearance of homologous and heterologous challenge isolates from the lungs. Overall, Peyer's patch immunization followed by an intratracheal boost was found to be highly effective, inducing both P6-specific CD4$^+$ T helper cell proliferation in mesenteric lymph node lymphocytes and increased antibody response in both serum and bronchoalveolar lavage fluid. There was a high correlation between bacterial clearance and recruitment of phagocytic cells to the lungs. The fact that the rate of enhanced bacterial clearance was highly variable among strains suggests that there were differences in immunologic defenses recognizing P6. The anti-P6 antibodies in sera and bronchoalveolar lavage fluid were cross-reactive, and the serum anti-P6 antibodies were bactericidal when assayed against homologous and heterologous isolates, even in the absence of enhanced pulmonary clearance. However, bactericidal activity of serum demonstrated in vitro did not correlate with protection in vivo. Overall, the data supported previous studies that indicated that P6 was a good potential vaccine candidate, able to afford protection against pulmonary infection by NTHi via induction of antigen-specific B and T cell responses in mucosal tissues.

DeMaria et al[45] immunized chinchillas with P6 and then challenged these animals either transbullarly or intranasally with a homologous isolate or a heterologous isolate, respectively. Fourteen days after transbullar challenge, the tympanic membrane inflammation scores were indistinguishable among cohorts; however, the number of chinchillas with ME fluid and the incidence of culture-positive ME fluids were reduced 48% and 51%, respectively, in P6-immunized chinchillas as compared to sham-immunized animals. The extent and duration of nasopharyngeal colonization was not affected by immunization with P6. All immunized animals demonstrated increased serum titers of anti-P6 antibody that was also bactericidal against the homologous and heterologous isolates. The finding that chinchilla anti-P6 antibody was bactericidal was in contrast to an earlier report by Green et al.[46] These data collectively demonstrated that while parenteral immunization with P6 did not protect against OM in this model or alter nasopharyngeal colonization, a level of protection was afforded in the form of augmented recovery from disease after direct challenge of the ME.

In addition to identification of potential NTHi vaccine candidates, several laboratories have begun to focus on methods of vaccination to induce the most protective immune response. Thus, different adjuvant formulations, delivery mechanisms, and dosing regimens have received attention as laboratories have attempted to optimize candidate vaccine formulations. Hotomi et al[47] investigated various dosing regimens for OMP P6 when delivered with the powerful mucosal and systemic adjuvant, cholera toxin B subunit. BALB/c mice that received an intranasal inoculation of P6 plus cholera toxin B subunit every 2 days for 2 weeks responded well at both the mucosal and systemic levels, with increased levels of P6-specific IgG and IgA in serum and IgA in nasopharyngeal washings. Conversely, intranasal immunization with P6 plus cholera toxin B subunit or with P6 alone twice at 2-week intervals induced weak systemic IgG and mucosal IgA responses. ELISPOT assay revealed anti–P6-specific antibody–producing cells in the nasopharyngeal mucosa of all animals immunized with P6 plus cholera toxin B subunit; however, the earliest appearance and greatest number of anti–P6-specific antibody–producing cells were detected in mice that were immunized every 2 days for 2 weeks. Unlike the study by DeMaria et al,[45] who found no effect on nasopharyngeal colonization after parenteral immunization of chinchillas with P6 plus complete or incomplete Freund's adjuvant, intranasal immunization of mice with P6 plus cholera toxin B subunit every 2 days for 2 weeks resulted in enhanced clearance of NTHi from the nasopharynx 3 days after challenge, as compared to 14 days for controls. However, there was no correlation between the level of anti–P6-specific antibody cells in nasopharyngeal washes and clearance of NTHi from the nasopharynx, since the animals immunized with P6 plus cholera toxin B subunit twice at 2-week intervals showed no enhanced clearance. The investigators also reported significant inhibition of adherence of NTHi to HEp-2 cells when NTHi were preincubated with supernatants of nasopharyngeal washes collected on day 21 from immunized animals in both groups that received P6 plus cholera toxin B subunit as compared to controls that received cholera toxin B subunit alone.

Badr et al[48] immunized mice of 3 different MHC class II haplotypes (H-2^d, H-2^k, H-2^s) with isolated P6, then measured levels of anti-P6 antibodies weekly for 68 weeks to determine the kinetics of the P6-specific response. They found that the primary immune response peaked 2 to 3 weeks after the initial injection of 40 μg of P6 and then steadily declined so that it reached an undetectable level at approximately 60 weeks. Although a strong, sustained primary antibody response could be demonstrated in all 3 strains of mice, the overall highest titers were elicited in BALB/c mice. The isotypes and subclasses of anti-P6 antibody were IgM, IgG1, IgG2a, and IgG2b; the IgG2b and IgG1 subclasses were dominant. Low levels of IgG3 were detected, but these did not increase with time. The investigators could elicit an immediate and strong secondary response in all 3 mice strains by intraperitoneal injection of a subimmunogenic dose of whole, live NTHi 62 weeks after the initial immunization. The antibodies to P6 were bactericidal when tested against 3 clinical NTHi isolates. These data further supported the role of P6 as an important vaccine candidate. OMP P6 is currently in phase I clinical trials.

Outer Membrane Protein 26. Kyd and Cripps[49] identified a novel 26 kd OMP (called OMP 26). Rats immunized via Peyer's patches with isolated native OMP 26 delivered in incomplete Freund's adjuvant, followed by an intratympanic boost 14 days later, were found to generate a significant dose-dependent anti–OMP 26 response in both serum (IgG, IgA, and IgM) and bronchoalveolar lavage fluid (IgG and IgA). Analysis of IgG isotypes in immunized rat serum indicated a predominantly Th1-type response. After a homologous or heterologous pulmonary challenge, OMP 26–immunized animals demonstrated significantly enhanced pulmonary clearance of both isolates as compared to nonimmune controls 4 hours after challenge. Clearance equivalent to that observed in animals immunized with killed whole bacterial cells was obtained in animals that received the higher dose of OMP 26. Greater absolute numbers of phagocytic cells were present in the bronchoalveolar lavage fluid of immune versus nonimmune animals, although differential counts for polymorphonuclear leukocytes and macrophages were the same. Polyclonal antiserum derived from mucosal immunization with OMP 26 was found to immunolabel intact NTHi cells, indicating the existence of at least 1 surface-exposed domain. The N terminal sequence of OMP 26 showed 100% homology with an export factor homolog in the Rd genome (HI 0916) and significant similarity to both the Skp transporter protein of *Pasteurella multocida* and OmpH of *Yersinia pseudotuberculosis*. The relationship of OMP 26 to these proteins or its potential role in extracytoplasmic protein transport is not yet known. OMP 26 was suggested as a potential vaccine candidate pending further studies to assess its degree of conservation and role on the bacterial surface.

El-Adhami et al[50] cloned and sequenced the gene encoding OMP 26. Analysis of the nucleotide sequence showed that the OMP 26 gene was 99% identical to the *ompH* gene in *H influenzae* strain Rd. Restriction fragment length polymorphism analysis indicated that this gene was relatively conserved among 20 isolates cultured from sputum, eye, vaginal, or throat swabs, whereas the translated amino acid sequence of OMP 26 of these strains showed similarities ranging between 96.5% and 99.5%. Eleven variants of the mature protein were identified. The investigators compared 2 recombinant forms of OMP 26 expressed in *Escherichia coli* to the native protein in ability to induce an effective immune response in a rat pulmonary challenge model. Both rOMP 26 (a 28 kd protein including the leader peptide) and rOMP 26M (a 26 kd protein lacking the 23 amino acid leader peptide) induced significant titers of IgG and IgA in serum and bronchoalveolar lavage fluid. However, the larger recombinant preprotein was found to be more effective in enhancing pulmonary clearance, and it induced a better cell-mediated immune response (determined by lymphocyte proliferation assay), as well as higher overall levels of systemic and mucosal antibodies. rOMP 26 was judged to be as effective as the native protein in this model. Both are being investigated further as vaccine candidates.

Pili. Since the previous Symposium, no studies have been published on the potential for the hemagglutinating pili of NTHi to serve as a vaccine candidate. Several detailed analyses of the pilus gene cluster of *H influenzae* have been conducted. These are reviewed in the Panel Report on microbiology and immunology.

Outer Membrane Protein P5 Fimbrin. Two synthetic chimeric peptides of P5 fimbrin (LB1 and LB2), derived from the third and fourth of 4 predicted surface exposed regions of this adhesin, were introduced at the previous Symposium as potential immunogens against NTHi-induced OM.[51] Since then, several studies attempting to further develop LB1 and its derivatives have been published. A recombinant fusion protein immunogen called LPD-LB1(f)$_{2,1,3}$ was designed and tested along with LB1, lipoprotein D (LPD), and a deacylated recombinant form of LPD (PDm) for relative efficacy versus homologous challenge in 2 active transfer immunization studies and 1 passive transfer immunization study.[52] Whereas neither LPD nor PDm protected against OM, both augmented recovery from disease. However, the 2 P5 fimbrin–based immunogens, LB1 and LPD-LB1(f)$_{2,1,3}$, significantly augmented clearance of NTHi from the ME and nasopharynx of chinchillas in the active protection studies. Moreover, antibodies directed against each of these immunogens, when delivered to adenovirus-compromised juvenile chinchillas, significantly protected against development of OM in the passive transfer protocol.

Webb and Cripps[53] further investigated the ability of specific epitopes of P5 fimbrin to function as protective antigens. They synthesized 3 peptides derived from predicted surface-exposed loops 1 and 4, as well as 1 derived from the C terminal of this OMP that has been predicted to be located in the periplasm. All 3 peptides contained an N-terminal T cell promiscuous epitope from measles virus fusion (MVF) protein. Peptides MVF/L1A and MVF/L4 (corresponding to the conserved motif of loop 1 and the central portion of loop 4, respectively) were not found to be efficacious in this model. However, peptide MVF/H3, homologous to the highly protective peptide 10 of OprF of *Pseudomonas aeruginosa*, was able to significantly enhance clearance of NTHi from the acutely infected rat lung. Immunization of rats with MVF/L4 via Peyer's patches followed by an intratympanic boost significantly reduced the number of viable NTHi in both bronchoalveolar lavage fluids (74%) and lung homogenates (70%) as compared to controls. Despite demonstrated efficacy, MVF/H3 elicited a poor antibody response. Protection was observed in the absence of detectable peptide-specific antibody. These data suggested that a cell-mediated, rather than a humoral, response played an important role in the observed enhanced clearance. Interestingly, 2 studies have now shown that chimeric synthetic peptides containing a B cell epitope derived from the fourth predicted surface-exposed region of P5 fimbrin plus a promiscuous T cell epitope of MVF does not elicit a protective immune response in either the rat, as discussed here, or in the chinchilla.[52] This region of P5 fimbrin, while highly conserved in NTHi isolates recovered from instances of acute disease,[54] is hypervariable in isolates from patients with chronic bronchitis.[55]

Kennedy et al[56] have continued to evaluate both LB1 and LPD-LB1(f)$_{2,1,3}$ as potential vaccine antigens, testing their breadth of protective efficacy against 3 NTHi isolates. The challenge strains were selected based on known amino acid sequence diversity in surface-exposed region 3 of P5 fimbrin that allows for segregation of NTHi into 1 major group and 2 minor groups. Thus, 2 of the 3 isolates were heterologous and 1 was homologous to the P5 fimbrin epitope contained in LB1. Conversely, LPD-LB1(f)$_{2,1,3}$ was specifically designed to accommodate this diversity, and thus, none of the 3 challenge strains was considered heterologous relative to this epitope for animals receiving antiserum directed against LPD-LB1(f)$_{2,1,3}$. Passive transfer of antiserum directed against both immunogens proved to be highly protective against OM induced by all 3 challenge isolates; however, total and early eradication of NTHi from the nasopharynx was not equivalent among strains. Both immunogens generated antibodies that showed preferential ability to induce clearance of the majority group 1 isolate from the nasopharynx over a group 2a or group 3 strain. Despite this selective clearance effect, all immune cohorts were protected from development of OM. Comparison of the relative colony-forming units of NTHi per milliliter of nasopharyngeal lavage fluids in sham versus immune cohorts indicated that in the majority of animals, simply reducing the bacterial load in the nasopharynx appeared to protect against OM in this model. Both P5 fimbrin–based immunogens continue to be investigated as vaccine candidates.

Lipoprotein D. Akkoyunlu et al[57] overexpressed the nonacylated form of protein D called PDm in *E coli* and demonstrated that 11% to 15% of affinity-purified human and rat antibodies recognized surface-exposed epitopes of protein D. Hyperimmunization of rats with PDm induced high levels of IgG and IgA antibodies in serum that had significant bactericidal activity against homologous and heterologous *H influenzae* isolates. Affinity-purified human anti-PDm antibodies were not bactericidal; however, the investigators speculated that this may have been due to the pH of the isolation conditions. This group later studied the local and systemic antibody response to protein D after immunization and challenge of rats in an NTHi and Hib AOM model.[58] In animals challenged and rechallenged in the ME with a type b isolate or with NTHi, IgG and IgA antibodies developed in serum, but not in ME lavage fluids or saliva. In contrast, oral — and, to a lesser extent, nasal — immunization with either NTHi or recombinant *E coli* that expressed protein D induced high levels of protein D–specific salivary IgA. However, there was no increase in antibodies to protein D in the ME lavage fluids or sera of oral or nasally immunized animals. Protein D was thus found to elicit variable local or systemic antibody responses, dependent on the route of delivery and form of administration.

In a more recent study,[59] immunization with PDm elicited bactericidal antibodies against homologous and heterologous NTHi isolates. Rats immunized with LPD had higher levels of serum antibodies (IgG and IgA) than did rats immunized with the nonacylated PDm. Neither LPD nor PDm prevented the development of AOM when rats were challenged intrabullarly with NTHi; however, 8 days after challenge, 50% of the LPD-immunized rats had recovered from AOM and 30% had negative ME cultures, compared to values of 30% and 0%, respectively, in PDm-immunized rats. Different concentrations of LPD and PDm were also conjugated to PRP to test their protective potential against Hib challenge. Two LPD-PRP conjugates and 1 PDm-PRP conjugate were found to induce significant protection against Hib-induced AOM, with the greatest protection conferred by the conjugate that incorporated the highest concentration of LPD. Overall, these data indicated that despite the ease of large-scale production of PDm, the acylated form of protein D is more immunogenic, and it induced an adjuvant effect when used as a carrier for PRP. Lipoprotein D and PDm have also been assessed in chinchilla models (see section on OMP P5 fimbrin). As in the rat model, both induced high-titer sera with bactericidal activity that did augment recovery from disease; however, neither prevented the development of OM.

Transferrin-Binding Protein B. Webb and Cripps[60] assessed the vaccine potential of a surface-exposed component of the NTHi human transferrin receptor, TbpB, and determined whether the antibody response elicited was cross-reactive with heterologous strains of NTHi. The efficacy of immunization with a recombinant form of TbpB (rTbpB) was determined by assessment of the pulmonary clearance of viable bacteria 4 hours after a live challenge with NTHi. There was a significant reduction in the number of viable bacteria in both the bronchoalveolar lavage fluid (34% for the 20-μg dose and 58% for the 40-μg dose) and lung homogenates (26% for the 20-μg dose and 60% for the 40-μg dose) of rats immunized with rTbpB as compared to the control animals. While the rTbpB-specific antibodies from the immunized rats were nonspecific in the recognition of TbpB from 6 heterologous NTHi strains on Western blots, these antibodies differed in their ability to block transferrin binding to heterologous strains and to cross-react in bactericidal assays. If bactericidal antibodies are key indicators of the efficacy of the immune response in eliminating NTHi, these data suggest that while immunization with rTbpB stimulates protective responses against the homologous isolate, variability in the recognition of TbpB from heterologous isolates may limit the potential of rTbpB as an NTHi vaccine component.

D15 Protein. Loosmore et al[61] cloned and sequenced the *d15* gene from 2 strains of Hib and 2 strains of NTHi. The nucleotide and deduced protein sequences of *d15* were highly conserved, with only a small variable region identified near the carboxyl terminus of the protein. Analysis of upstream sequences revealed that the *H influenzae d15* gene may be part of a large potential operon of closely spaced open reading frames, including one with significant homology to the *E coli cds* gene encoding CDP–diglyceride synthetase. Southern blot analysis demonstrated that the *d15* gene is also present in *H influenzae* types a (Hia), c, d, e, and f and in *Haemophilus parainfluenzae*. A recombinant D15 (rD15) protein was expressed in good quantity in *E coli* from the inducible T7 promoter, and monospecific anti-rD15 antibodies were raised. Immunoblot analysis of *H influenzae* serotypes a, b, c, d, e, and f, NTHi, and *H parainfluenzae* lysates revealed that they all expressed a cross-reactive D15-like protein. Purified rD15 was found to be highly immunogenic in mice, guinea pigs, and rabbits, and passive transfer of anti-rD15 antibodies protected infant rats from challenge with Hib or Hia in infant rat models of bacteremia. Thus, D15 is a highly conserved antigen that is protective in animal models, and it may be a useful component of a universal subunit vaccine against *Haemophilus* infection and disease.

Yang et al[62] sought to further characterize the protective epitopes on this protein. A 20 kd N terminal fragment of D15 protein (truncated D15 [tD15]) was expressed as a fusion protein with glutathione *S*-transferase in *E coli.* The tD15 moiety was cleaved from glutathione *S*-transferase by means of thrombin and purified to homogeneity. The purified soluble tD15 appeared to contain immunodominant protective epitope(s) against Hia and Hib, since rabbit antisera directed against tD15 were capable of protecting infant rats from Hia or Hib bacteremia. The ease of purification of soluble tD15, therefore, makes it a better candidate antigen than full-length recombinant D15, which is produced as inclusion bodies in *E coli.* Further, both the purified tD15 fragment and a mixture of tD15-derived peptides spanning amino acid residues 93 to 209 of the mature D15 protein were capable of inhibiting the protection against Hib conferred on infant rats by rabbit anti-tD15 antiserum; this finding indicates that the protective epitopes of D15 may not be conformational.

HMW1/HMW2 Proteins. The HMW1/HMW2-like proteins are highly immunogenic high–molecular weight proteins previously identified as important in adherence of NTHi to human epithelial cells. Barenkamp and St Geme[63] determined whether immunization with 2 such proteins, HMW1 and HMW2, purified from prototype NTHi strain 12, would modify the course of experimental OM caused by the homologous strain. Chinchillas immunized subcutaneously with an HMW1/HMW2 protein mixture in Freund's adjuvant were challenged by transbullar inoculation. Infection developed in 5 of 5 control and 5 of 10 immunized animals ($p = .08$). Bacterial counts in ME fluid specimens were significantly greater in control animals than in immunized animals ($p = .014$, Mann-Whitney U test). Serum antibody titers following immunization were comparable in uninfected and infected animals. However, infection in immunized animals showed a uniform association with the appearance of bacteria down-regulated in expression of the high–molecular-weight proteins, suggesting bacterial selection in response to immunologic pressure. Although protection following immunization was incomplete, these data suggest that the high–molecular-weight adhesion proteins are potentially important protective antigens that might represent one component of a multicomponent NTHi vaccine.

HtrA/H91A Protein. Loosmore et al[64] characterized the *htrA* gene of NTHi, and cloned and sequenced the genes from 2 strains. The encoded, approximately 46 kd HtrA proteins were found to be highly conserved. The *H influenzae* HtrA had approximately 55% identity with *E coli* and *Salmonella typhimurium* HtrA stress response proteins, and expression of the *H influenzae htrA* gene was inducible by high temperature. The recombinant HtrA (rHtrA) was expressed in *E coli*, and the purified protein was found to have serine protease activity. The rHtrA was found to be very immunogenic and partially protective in both the passive infant rat model of bacteremia and the active chinchilla model of OM. Immunoblot analysis indicated that HtrA is antigenically conserved in encapsulated and nontypeable *H influenzae* species. Site-directed mutagenesis was performed on the *htrA* gene to ablate the endogenous serine protease activity of wild-type HtrA, and it was found that 8 of 9 recombinant mutant proteins had no measurable residual proteolytic activity. Two mutant proteins were tested in the animal protection models, and one, H91A, was found to be partially protective in both models. H91A HtrA may be a good candidate antigen for a vaccine against invasive Hib disease and OM and is currently in phase I clinical trials.

Detoxified Lipooligosaccharide. Gu and Sun[65] reported that detoxified lipooligosaccharide (LOS)–protein conjugates from NTHi elicited a significant rise of anti-LOS antibodies with bactericidal activity in rabbits. They evaluated whether vaccination with the conjugates would protect against NTHi OM in chinchillas. Chinchillas received 3 subcutaneous or intramuscular injections of detoxified LOS–conjugated tetanus toxoid, detoxified LOS–conjugated high–molecular-weight proteins from NTHi, or saline in Freund's adjuvant and then were challenged by transbullar inoculation of NTHi. All vaccinated animals responded with elevated serum titers of anti-LOS antibody, and 49% demonstrated bactericidal activity against the homologous strain. Otitis media with culture-positive NTHi effusions developed in all controls and 56% of the vaccinated animals ($p < .001$). Bacterial counts in ME fluids were lower in the vaccine groups than in controls. The incidences of infection in, respectively, the unchallenged ear and the inner ear were 26% and 28% in the vaccine groups and 53% and 58% in the controls ($p < .05$). Signs of infection observed by otoscopy were less severe in the vaccine groups than in the controls. There was no significant difference between the 2 vaccine groups. These data indicate that active immunization with LOS-based conjugates reduces the incidence of NTHi-induced OM in the chinchilla model.

MORAXELLA CATARRHALIS VACCINES

Moraxella catarrhalis has been recognized as an important ME pathogen only in the last decade. Therefore, work on this bacterium lags behind that on *S pneumoniae* and NTHi. However, several OMP antigens with potential as vaccine candidates had been identified in 1995.[41] A significant obstacle in evaluating vaccine antigens for *M catarrhalis* is lack of reliable correlates of protection from infection. Animal models of OM, including the chinchilla, induced by *M catarrhalis* have been disappointing because of rapid clearance of the inoculum from the ME. A mouse pulmonary clearance model has been developed and used, but the results must be interpreted with caution because of rapid clearance of the organism and absence of disease.[66,67] An important area of research identified in 1995 was describing an in vitro correlate of protection of *M catarrhalis* ME infection. There is little definite evidence that bactericidal antibody to *M catarrhalis* is associated with protection from OM.

Detoxified LOS conjugates induced significant immune responses in rabbits, and conjugate-induced antisera had complement-mediated bactericidal activity against the homologous strain and heterologous strains of *M catarrhalis*.[68] Monoclonal antibody (Mab) 11C6, which reacts to a surface-exposed epitope of OMP B1 expressed by *M catarrhalis* 7169, was developed, and the bactericidal activity was determined.[69] The results suggested that OMP B1 is a potential vaccine antigen against *M catarrhalis* infections. Further analysis with Mab 11C6 revealed the presence of this OMP B1 epitope on 31% of the clinical isolates tested.

MUCOSAL DELIVERY OF VACCINES

Intranasal immunization can induce mucosal, as well as systemic, immune responses and might be a useful regimen for vaccination against OM. An intranasal spray of attenuated influenza virus vaccine significantly reduced culture-positive influenza (14 cases among 1,070 subjects) as compared to placebo (95 cases among 532 subjects).[70] Further, the incidence of febrile OM was 30% lower among vaccine recipients.

Intranasal immunization with NTHi OMP P6 and cholera toxin evoked a mucosal IgA response, as well as a systemic IgG response, against P6. Immunization enhanced nasopharyngeal clearance of NTHi and inhibited NTHi adherence to cultured human epithelial cells.[47] In another experiment, mice were immunized nasally, orally, intratracheally, or intraperitoneally with NTHi antigen together with cholera toxin.[71] Antigen-specific IgA responses in nasal passages were highest in nasally immunized mice. Intranasal immunization also induced serum IgG responses in serum. Cytokine analysis showed that Th2- and Th1-type cells were generated after the immunization. Further, bacterial clearance of a homologous strain of NTHi from the nose was significantly enhanced in the nasal immunization group. Mutant cholera toxin has been developed as a nontoxic mucosal adjuvant and induces Th2-type responses.[38,72] Intranasal vaccination might be a useful vaccine delivery system to prevent OM.

INFLUENZA VACCINES

At present, influenza vaccine is the only commercially available vaccine for the control of respiratory virus infections. The current preparations licensed for use in children are inactivated split-virus vaccines, which are administered intramuscularly and can be given to children older than 6 months of age. These vaccines are relatively safe, with minimal side effects. In children younger than 9 years, 2 doses of the vaccine administered 1 month apart are recommended for the initial immunization and are believed to produce an adequate antibody response.

Three randomized, controlled clinical trials have demonstrated the efficacy of influenza vaccine for prevention of AOM. In the first of these studies, conducted among 374 children in day care in Finland, influenza vaccine was administered to half of the children before the expected influenza outbreak, with an equal number of unvaccinated children serving as controls.[73] During the following influenza A epidemic, the incidence of AOM associated with influenza A was decreased by 83% in the vaccinated children. There was a 36% reduction in the overall incidence of AOM in the vaccine group as compared with the controls. Essentially similar results were obtained in a subsequent study in the United States.[74] Among 186 children attending day care, influenza-vaccinated children had 32% fewer episodes of AOM during the influenza epidemic than control children.

A recent study investigated the efficacy of a newly developed live attenuated intranasal influenza vaccine in 1,602 children.[70] While the efficacy of this intranasally administered vaccine was 93% against culture-confirmed influenza, the vaccinated children had 30% fewer episodes of febrile AOM. During the first 10 days after the administration of the vaccine, more children in the influenza vaccine group had rhinorrhea, nasal congestion, or fever as compared with the placebo recipients. However, these symptoms were minor and short-lived, and development of AOM due to vaccination with live virus was not reported in any child.

Results of the influenza vaccine trials indicate that prevention of viral infection, which usually precedes AOM, is an effective way to prevent the development of AOM. An obvious inference is that effective vaccines against other major viruses predisposing to AOM — especially against respiratory syncytial virus (RSV) — could have a substantial effect on the incidence of AOM in children. Nonetheless, in developing live viral vaccines to be used for prevention of AOM, it is important that such vaccines are sufficiently attenuated, so as not to induce the development of AOM as an adverse outcome of the vaccination.

RESPIRATORY SYNCYTIAL VIRUS VACCINES

Development of active vaccines against RSV infection continues.[75,76] The live virus vaccines undergoing clinical testing include temperature-sensitive mutants and cold passage–adapted strains. The subunit vaccines undergoing clinical testing include purified fusion proteins (PFP-1 and PFP-2). While these vaccines have been found to be relatively safe, they do not seem to induce protection against the development of lower airway disease in infants and young children. These vaccines have not been evaluated for protection against OM or for development of OM caused by the intranasally administered mutant vaccine viruses themselves. At the moment, it seems unlikely that a vaccine without lower airway protection would be evaluated for protection against OM alone.

Vaccines against RSV may provide protection of the ME by inhibiting RSV replication in the nasopharynx and subsequent invasion of the virus into the ME, as well as by reducing the development of conditions necessary for bacterial superinfection. Data from the recently approved passively administered antibody vaccines effective against RSV lower airway disease suggest that inhibition of RSV infection alone may not be protective for the ME. An RSV-enriched immune globulin derived from pooled human plasma, RSV-IVIG (Respigam), given intravenously every month during the winter, provided modest protection against OM.[77] However, the humanized monoclonal antibody vaccine palivizumab (Synagis), given intramuscularly every month during the winter, failed to show a similar benefit.[78] It is very likely that the protective effect of RSV-IVIG was due to antibacterial antibodies that were present singly or in combination with RSV antibodies. Further studies are needed, because the palivizumab trials included a very small number of children with OM, and infectious agents were not identified. The prohibitive cost of these passive antibody preparation vaccines would preclude their use specifically for protection of the ME.

In the context of OM, it is not known whether RSV-specific cellular immunity generated by active RSV vaccines would provide better protection than passive polyclonal or monoclonal antibodies. There is also a formidable challenge in designing appropriate clinical trials of RSV vaccine efficacy against OM. For vaccines against bronchiolitis, it has been estimated that vaccines with 60% efficacy would require 1,500 infants enrolled in a clinical trial to achieve an 80% power.[79] Similar sample size estimates for OM vaccine trials are unavailable.

PARAINFLUENZA VACCINES

Parainfluenza virus types 1, 2, and 3 are major causes of laryngotracheobronchitis and the third major group of viruses causing OM. Development of vaccines against parainfluenza would need to target all 3 types of viruses, since the 2 surface glycoproteins against which protective neutralizing antibodies are produced, fusion protein and hemagglutinin-neuraminidase protein, are antigenically distinct.[80] While no candidate parainfluenza vaccines have been evaluated for protection against the development of OM, a variety of vaccines have been tested in animal models for protection against upper and lower respiratory tract disease. Only 2 vaccines have been evaluated in humans: an intranasal cold-passaged (temperature-sensitive) mutant strain of human parainfluenza virus type 3 and an intranasal bovine parainfluenza virus type 3.[81-83] Both of these vaccines have been found to be relatively safe and immunogenic in infants. However, there are insufficient data on the protective benefits against respiratory disease. A major remaining obstacle to successful immunization of infants against parainfluenza virus–associated disease may be the relatively poor immune response of very young infants to primary virus infection. Clinical trials of vaccines evaluating their benefit in the lower airway will need to observe for effect on OM, as well.

GOALS

1. Test RSV, parainfluenza, and influenza vaccines for AOM protection.

2. Standardize serologic measurement of antibody and opsonophagocytic activity against pneumococcus to permit evaluation of the serologic correlates of protection afforded by the new pneumococcal polysaccharide–protein conjugate vaccine.

3. Continue clinical trials to evaluate the efficacy of different formulations of pneumococcal conjugate vaccines in preventing AOM in infants, and carry them out in different populations and in different geographic areas. Enhance and prolong the immunogenic and protective effects of these vaccines. Study the added value of incorporating pneumococcal surface proteins in conjugate vaccines.

4. Identify in vitro correlates of protection against pneumococcal OM.

5. Test NTHi antigens in humans. P4, P6, detoxified LOS–tetanus toxoid, and Hin47 are in phase I

trials. HMW1/HMW2, P5 fimbrin, and protein D are nearly ready for phase I trials.

6. Establish in vitro correlates of a protective immune response to NTHi.

7. Characterize potential vaccine antigens of *M catarrhalis* and proceed to human testing after purity and safety are documented.

8. Develop and test hyperimmune globulins against OM pathogens. Their usefulness in children with normal, as well as compromised, immune status should be evaluated and compared with that of other preventive approaches.

9. Establish that protective IgG antibodies against pneumococcus, NTHi, and RSV could be provided by maternal immunization. Initial studies should establish safety and immunogenicity of maternal immunization with relevant antigens, and transplacental passage of protective antibodies. Ultimately, a maternal immunization efficacy trial should be performed.

10. Explore the usefulness of new vaccine adjuvants in enhancing the immune response to vaccine antigens.

11. Continue investigative mucosal delivery systems for OM vaccines: viral, NTHi, *M catarrhalis*, and pneumococcal PspA.

12. Determine whether new vaccine technology developed in other areas can be applied to the prevention of OM (eg, recombinant proteins, naked DNA).

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8. DIAGNOSIS AND SCREENING

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INTRODUCTION

Middle ear effusion (MEE) is caused by episodes of acute otitis media (AOM), as well as otitis media with effusion (OME). An episode of AOM usually manifests signs and symptoms of an acute infection, while episodes of OME are often "asymptomatic."

Recurrent or persistent episodes of MEE cause recurrent or prolonged periods of hearing loss, which may have adverse effects on speech, language, development, and balance for some children. Thus, it is essential that sensitive and specific diagnostic and screening methodologies be available to identify children with MEE who may be at risk for complications and sequelae of the disease.

PNEUMATIC OTOSCOPY

Restricted mobility of the tympanic membrane is an important sign of MEE. Thus, otoscopy alone is insufficient for the detection or diagnosis of MEE. Pneumatic stimulation significantly enhances the accuracy of otoscopy and is strongly recommended. It is important to choose a correct-sized speculum in order to get an airtight seal to allow application of positive and negative pressures in the ear canal to assess the movements of the tympanic membrane. Also, the removal of cerumen is mandatory to maximize the visualization of the tympanic membrane, in order to examine its position, color, and mobility. An excellent review of pneumatic otoscopy for the diagnosis of AOM is provided by Pelton.[1] When performed by trained and experienced clinicians, pneumatic otoscopy and tympanometry appear to be equivalent techniques for the identification of MEE.[2] Less experienced practitioners, not validated in the use of pneumatic otoscopy, should incorporate tympanometry for the identification of MEE. It should be kept in mind that a normal-appearing tympanic membrane does not preclude the presence of middle ear pathology.[3]

ACOUSTIC IMMITTANCE

Instruments for the measurement of acoustic immittance are useful in clinical practice, essential for clinical research, and necessary for large-scale screening. Acoustic immittance instruments are used to record tympanograms, measure acoustic (middle ear muscle) reflexes, and perform tests of eustachian tube function. More limited instruments (tympanometers) perform only tympanometry and are well suited for screening programs and busy clinical practices.

Conventional Tympanometry. Tympanometry is a measure of the acoustic admittance of the ear as a function of ear canal air pressure. Four features of the tympanogram are useful for evaluating middle ear function: static admittance, tympanometric gradient and width, tympanometric peak pressure, and equivalent ear canal volume. Quantitative measurements, as opposed to qualitative descriptors (simple typing of tympanogram shape), are recommended for the measurement and description of middle ear function. These allow changes in middle ear function to be accurately described and avoid use of confusing or unspecified criteria for distinguishing among tympanograms.[4-6] Nozza et al[7] examined the sensitivity and specificity of tympanometric features, alone and in combination with otoscopy, for children with normal middle ear function, those with histories of chronic otitis media, and those with surgically confirmed MEE. Tympanometry with quantitative pass-fail criteria was equivalent to pneumatic otoscopy when performed by highly skilled, validated otoscopists. The use of both pneumatic otoscopy and tympanometry for diagnosis of otitis media in children over 4 months of age is recommended. Normative quantitative tympanometric data are available for children[5,6] and infants.[8]

Fields et al[9] and Ovesen et al[10] showed that the sensitivity and specificity obtained by general practitioners using a screening tympanometer were similar to the results obtained by audiologists using a more sophisticated instrument. A portable minitympanometer is also a feasible alternative, although in young and uncooperative children, its specificity is poor.[11]

Gaihede[12] suggests that the detection of negative middle ear pressure may lead to an overdiagnosis of MEE in young children because of the small volume of the mastoid air cell system. Another study suggests that supine bottle-feeding has a significant effect on middle ear pressure that can also result in atypical peak pressure values.[13]

There is a paucity of data on the use of tympanometry in the diagnosis of MEE in infants 4 months of age and younger and in newborns. However, existing evidence suggests that conventional (226 Hz) tympanometry is not an effective test for middle ear disease in young infants.[5,6]

Multifrequency and High-Frequency Tympanometry. While conventional (226 Hz) tympanometry is not effective for detection of MEE in infants 4 months of age and younger, there is some evidence that tympanometry with higher probe frequencies may be more valid for detecting MEE.[14-16] The use of probe tones ranging from 226 to 2,000 Hz and the use of a single high-frequency probe tone (eg, 1,000 Hz) are protocols that show promise for the development of a sensitive and specific tympanometric test for detection of MEE in young infants. In an animal study of experimental middle ear disorders mimicking otitis media sequelae, multifrequency tympanometry was significantly better than single-frequency tympanometry for identification of middle ear disease.[17]

Acoustic Reflectometry. The acoustic reflectometer is a commercially produced instrument designed to detect MEE. The instrument delivers a complex acoustic stimulus to the ear canal and measures the frequency spectrum of the signal at the point of delivery (lateral to the entrance to the ear canal, within the instrument). The measured signal is dependent on the unspecified acoustic characteristics of the device itself, the acoustic characteristics of the ear canal, and the mechano-acoustic characteristics of the tympanic membrane. Because tympanic membrane mechanics are altered by middle ear disease, the presence of disease alters the measured sound characteristics.

The current version of the device uses an algorithm for distinguishing normal from abnormal ears called "spectral gradient reflectometry." When the ear canal is terminated by high impedance — as in an ear with MEE — there is a sharp null in the spectral characteristic of the signal recorded within the device. The sharpness of the null is predictive of ear disease: the sharper the null, the higher the likelihood of MEE. The manufacturer provides a scale relating the sharpness, expressed in degrees, to the likelihood of MEE.

Because the instrument is not calibrated to measure ear characteristics, independent of the characteristics of the device, the measurements are not useful for quantifying the mechano-acoustic effects of ear disease. Interpretation of results is limited to a qualitative assessment of differences between normal and diseased ears and does not provide useful assessment of the mechanical properties of the ear. Because the device was designed to perform a screening rather than a measurement, its usefulness is exclusively dependent on the rather arbitrary selected response characteristics on which the prediction of effusion is based.

While several studies have addressed the clinical usefulness of the instrument,[18-21] the efficacy of acoustic reflectometry has *not* been demonstrated to be adequate in professional hands,[22] nor to be equal to or superior to tympanometry. Importantly, the instrument is uncalibrated, and the current instrument cannot be calibrated.

Wideband Reflectance Measures. Wideband reflectance appears to be a promising technique for the detection of MEE and conductive hearing loss mainly caused by MEE.[5,6,23-25] Unlike conventional acoustic immittance measures that are restricted to low-frequency (<2 kHz) regions, measurement of relative power absorption in the ear canal may allow the identification of subtle conductive disorders across the frequency range important for speech perception (up to 5 kHz). Wideband reflectance measures may have great potential in hearing screening programs for children — particularly in newborns — for detection of transient middle ear disorders. Newborns and very young infants (less than 4 months of age), in particular, are a population for whom conventional (226 Hz) acoustic immittance measures have not been sufficiently specific or sensitive for use in comprehensive follow-up when the infants fail evoked otoacoustic emission (OAE) or auditory brain stem (ABR) response screening before hospital discharge.

EVOKED OTOACOUSTIC EMISSIONS

Because MEE affects both forward and reverse transmission through the middle ear, OAEs are usually absent or reduced in amplitude in the presence of MEE.[26] Since some ears with MEE have minimal hearing loss, the presence of MEE reduces the specificity of OAEs as a screening technique for detection of significant hearing impairment. However, OAEs may be effective as a combined screen for middle ear disease and sensorineural hearing loss. Nozza et al[27] reported that OAEs were effective as a combined screen for hearing loss and MEE, with sensitivity and specificity ranging from 0.8 to 1.0 for various pass-fail criteria. Some studies suggest that the band reproducibility of transient evoked OAEs below 2 kHz may provide a method for tracking the resolution of MEE.[28] Distortion product OAEs were used in a pilot screening project with infants and toddlers 0 to 36 months of age who were at risk for both OME and hearing loss.[29]

AUDIOMETRY

Visual reinforcement audiometry (VRA) is now widely used for the comprehensive audiological assessment of threshold sensitivity in infants and toddlers (approximately 5 to 24 months of age). One study used VRA procedures to classify the hearing of infants and young children (at and below 2 years of age) according to each child's 3-frequency (500, 2,000, and 4,000 Hz) audiogram.[30] In another study,[31] infants as young as 5 months of age could be assessed reliably by a computer-assisted VRA procedure. Milder degrees of hearing loss associated with some cases of MEE were delineated by this audiometric method.

Chronic MEE,[32] as well as a history of recurrent AOM,[33] has been shown to produce hearing loss in the extended high-frequency region (8 to 20 kHz). These high-frequency hearing losses are related to disease duration and severity and do not appear to be related to persistent middle ear disease. A subsequent study[34] demonstrated that wideband middle ear impedance was unrelated to high-frequency hearing loss, supporting a cochlear rather than a middle ear etiology.

Behavioral audiometry provides the clinician with the opportunity to observe the appropriateness of an infant's or young child's response to sound. In addition, audiometric screening and assessment of hearing allows mixed hearing loss (combined conductive loss and sensorineural loss) to be detected. The latter benefit is not available when acoustic methods (tympanometry, reflectance, OAEs) sensitive to middle ear disease only are used to screen for MEE exclusively. Such technologies provide no information about hearing status.

ELECTROPHYSIOLOGY

Auditory brain stem response testing is often used to assess infants and children who cannot be tested by conventional behavioral techniques. Air- and bone-conducted ABR techniques are useful in distinguishing cochlear impairments from the conductive hearing loss associated with MEE. Frequency-specific ABR procedures are particularly useful for delineating auditory sensitivity across the speech-frequency range. Click-evoked ABR responses can miss significant, frequency-specific hearing loss accompanied by otitis media or other ear disorders.

Conventional ABR testing has some limitations for screening of newborns. It involves relatively long test times, the equipment is expensive, and trained audiologists are needed. The recent development of automated screening equipment for ABR testing has addressed these concerns. The most widely used automated ABR screener, the ALGO (Natus Medical, San Carlos, California), can be used by a variety of personnel. A built-in artifact rejection feature halts data collection if testing conditions are unfavorable. The electrodes and earphones are designed for easy application, and a pass-or-refer result is automatically provided at the end of each test. Combined data from several studies that compared the ALGO to conventional ABR equipment showed the specificity of the ALGO to be 96% and the sensitivity 98%.[35,36]

TYMPANOCENTESIS AND MYRINGOTOMY

Tympanocentesis and myringotomy is not commonly used in clinical practice as a screening method. Because of the recent development of resistant bacterial strains, it has become increasingly more important as a diagnostic tool. With tympanocentesis, the presence and type of MEE can be determined. In addition, material can be obtained for microbiological evaluation to guide in the management of the disease.[37] Myringotomy is also required in some patients to provide drainage of the middle ear or for pain relief.

IMAGING

For imaging of the temporal bone, the more traditional methods are computed tomography with and without contrast enhancement and magnetic resonance imaging with gadolinium. Bony abnormalities of the temporal bone are demonstrated primarily with computed tomography and include demineralization and erosive and destructive lesions. Magnetic resonance imaging is best for imaging of soft tissue structures in the middle ear, mastoid process, and central nervous system. These methods are not used for routine diagnosis, but are used to rule out complications of otitis media such as mastoiditis and cholesteatoma.

SCREENING

Several high-risk groups of infants may require specific screening for MEE (and associated hearing loss). These include neonates cared for in the neonatal intensive care unit (NICU); infants and young children with cleft palate, Down syndrome, or other craniofacial disorders; and infants and children with acquired immunodeficiency syndrome. In addition, infants and preschool children (birth to 36 months) who attend group day care, who are exposed to passive smoke, who are bottle-fed, and/or who have a history of recurrent AOM may also be at risk for MEE. It is possible that screening for MEE and hearing loss in day care facilities and preschools would be beneficial in detecting recurrent disease and associated persistent conductive hearing loss in young children.

Neonates. Interest in universal newborn hearing screening continues to grow. The differentiation of neonates with positive screening outcomes due to MEE or other middle or external ear conditions versus infants with cochlear hearing loss will become increasingly important. In addition to their increased risk for sensorineural hearing loss, high-risk infants cared for in the NICU are at greater risk for MEE than well babies because of various medical conditions (eg, sepsis) and treatment factors (eg, nasotracheal intubation). Sutton et al[14] estimated that 29% of 84 NICU infants had MEE. They found that infants who were in the NICU for 30 days or more were at about a 4-times-greater risk for MEE than were infants who were discharged from the NICU sooner. Differentiating the potential cause of an in-hospital positive screening outcome could be accomplished by using a combination of middle ear and hearing screening technologies or using an alternative screening technology (eg, ABR testing).

Currently, it is unknown whether infants who fail hearing screening because of MEE are at greater risk for development of recurrent OME in childhood than newborns with normal middle ear function.

Middle ear effusion is recognized as a primary reason for failure of the neonatal hearing screening. Doyle et al[38] studied 200 healthy newborns who were 5 to 48 hours old by using pneumatic otoscopy, EOAE, and screening ABR technology. The pass rate for screening ABR when tympanic membrane mobility was normal was 93.6%, while it was 50% for ears with MEE. Ears classified by pneumatic otoscopy as normal had an 88% pass rate for EOAE screening, while in those identified with MEE, only 38% passed EOAE screening. Doyle et al[38] suggested that combining screening ABR and EOAE technologies was a sensitive screening protocol for the detection of MEE in neonates.

Sutton et al[14] obtained 678-Hz admittance tympanograms from 85 infants in a special care baby unit–NICU. They found tympanogram type (broadly classified) to be strongly associated with EOAE screening outcome. The categories of negative middle ear pressure and a flat tympanogram were considered evidence of abnormal middle ear status. McKinley et al[15] studied healthy newborns less than 24 hours old. Two-component admittance (susceptance and conductance) tympanograms were recorded for 226-, 678-, and 1,000-Hz probe frequencies. Rhodes et al[16] reported that 1-kHz-frequency tympanometry may be useful for detection of MEE in newborns.

Infants and Toddlers. Although behavioral audiometric screening has been used extensively with preschool (4 to 6 years) and school-age (6 years and above) children,[39] only recently has behavioral audiometry been used for the identification of infants and toddlers (6 to 36 months of age) with mild hearing loss associated with MEE. Several reports indicate that infants' hearing thresholds can be assessed reliably with the operant head-turn procedure known as visual reinforcement audiometry (VRA).[31] Recently, a computer-assisted behavioral screening algorithm (Classification of Audiograms by Sequential Testing: CAST), which incorporates Bayesian mathematical decision theory, has been demonstrated to be useful for detection of the mild degrees of hearing loss associated with MEE in infants and young children.[40] The screening algorithm is developmentally appropriate, bias-free, and time-efficient. The CAST results are classified into 1 of 9 hearing loss categories or may be considered broadly in a pass-fail classification scheme.[40] The limitation of this behavioral screening technique is that currently, the instrumentation is not widely available in clinical facilities.

Preschool and School Children. Detection of MEE may be best accomplished in schools, preschools, and day care settings by means of a combination of pure tone audiometry and tympanometry.[39] However, only limited data are currently available on the practical application and performance of these screening procedures in very young children. Pure tone screening in young children (beginning at about 30 months of age) can be readily accomplished with a play audiometry procedure. Use of tympanometry alone is not recommended for screening programs for young children. Detection and prolonged treatment of OME without the benefit of an audiological evaluation in an infant or preschool child has been cited as a reason for the late identification of infants and children with permanent sensorineural hearing loss.[41] More research is needed on the use of OAEs in hearing screening programs for identification of the hearing loss associated with MEE versus cochlear hearing loss.[27,29]

There are published referral guidelines for infants and children who fail hearing screening programs. In particular, programs identifying infants and children at high risk for MEE should have a protocol in place for repeat screening and referral as necessary.[39,42]

SCREENING INSTRUMENTS

Any screening instruments (audiometry, tympanometry, pneumatic otoscopy, acoustic reflectometry, EOAE) used for the detection of MEE should have demonstrated sensitivity and specificity and proven safety and effectiveness. Test instruments that are easily used, objective, and provide unambiguous outcomes (pass-refer) are needed for large-scale screening efforts in at-risk populations. Screening instruments are needed that are lightweight, handheld, and easily manipulated, have extended battery life, can provide hard copy or digital archiving of results, and can be readily and regularly calibrated. For current and future instruments, performance data for various target populations are needed. Finally, information on the practical aspects (test time, comfort level, and maintenance) of each technology is needed.

SPEECH AND LANGUAGE SCREENING

Infants and children with MEE who are at risk for communication delay[43] should be screened with age-appropriate and developmentally appropriate language screening instruments such as the Early Language Milestone Scale, the Clinical Linguistic and Auditory Milestone Scale, and/or parent checklists of language milestones. These screening procedures only take a short time to perform, and physicians should be familiar with these tests. Further studies are needed in this area; to our knowledge, no new screening tools for developing communication have been devised in the last 5 years.

TRAINING PROGRAMS

Both pneumatic otoscopy and tympanometry are routinely used in the diagnosis of MEE. Both techniques can be highly sensitive and specific in the diagnosis of MEE when used by trained and experienced clinicians. Both procedures require formal training in order to be practiced reliably, efficiently, and safely, and have been shown to improve the diagnostic skills of general practitioners.[44-46] An important contribution made during the past 4 years is the effort that has been made in developing training programs for instruction of physicians and nurse practitioners in the use of pneumatic otoscopy and tympanometry.[44] Training programs that incorporate videotaped instruction and video-otoscopy,[47] as well as the workshop format, are proving to be important in enhancing the abilities of general practitioners in detection of MEE. However, studies examining the effectiveness of such programs in achieving their stated goals are necessary.

PARENTAL IDENTIFICATION OF OME AND HEARING LOSS

Several studies[48-50] have found that parents are not accurate in their identification of either MEE or the hearing loss associated with OME in their children. These studies are particularly compelling, because parents and caregivers are frequently asked for their opinion regarding the presence or absence of hearing loss or ear infection. However, because parents are not able to detect reliably the hearing losses associated with MEE, the use of subjective judgements as a screening technique for identifying OME and hearing loss in the clinical setting is discouraged. Studies using acoustic reflectometry[51] and tympanometry[52] have shown that in-home monitoring is feasible. Potentially, more thorough education and training of parents, as well as the development of sensitive, objective screening tools for home use, may increase parents' abilities to promptly identify MEE and hearing loss in their children.

RESEARCH PRIORITIES

The long-term research priority in screening and diagnosis is to develop and validate cost-effective identification programs for populations that are at high risk for MEE and its associated hearing loss. In order to achieve that goal, the following short-term priorities need to be addressed.

The explosive growth of newborn hearing screening programs has produced an urgent need for an effective screening tool for identifying middle ear disorders in newborns. Handheld, high-frequency tympanometers and wideband reflectance instruments need to be developed and validated for this purpose.

Algorithms and scoring systems for the detection of MEE and hearing loss need to be developed and evaluated for use across infancy and childhood.

Training programs for the accurate diagnosis of middle ear disease and methods of educating primary care physicians about otitis media and its potential medical and developmental consequences need to be formally evaluated.

The relationships among OAEs, admittance measures, hearing/auditory tests, pneumatic otoscopy, and middle ear disease in infants and young children need to be more thoroughly examined. This information is necessary to establish the efficacy of any one technique or combination of techniques in detection and diagnosis of OME and any accompanying hearing disorder.

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9. TREATMENT, COMPLICATIONS, AND SEQUELAE

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INTRODUCTION

Safe and effective treatments and methods of preventing otitis media (OM) are desired goals of all who have an interest in this common disease. The Panel on Treatment convened at this Research Conference has elected to review the relevant research progress on OM published during the preceding 4 years (1995 to 1999) and the Reports that addressed its complications and sequelae. Adding a review of the literature related to the complications and sequelae at this time is appropriate, since there is a current lack of knowledge concerning most aspects of management of these conditions. We have also included the goals for future research related to OM and its complications and sequelae identified by Panel members at this and past Research Conferences. We hope this document will stimulate appropriately conducted research into these disease entities and the many unanswered questions that remain related to acute OM (AOM) and OM with effusion (OME).

ACUTE OTITIS MEDIA AND OTITIS MEDIA WITH EFFUSION

As recommended by Co-Chair Richard M. Rosenfeld, MD, MPH, and enthusiastically embraced by the other Panel members, reports published in the literature during the previous 4 years that relate to OM were ranked by criteria for evidence-based medicine.[1] This ranking system is different from summaries published by these Panels on Treatment in the past, and provides a useful technique for ranking the results of published clinical trials. Definitive randomized controlled trials (RCTs) or meta-analyses of appropriate design are the highest level, and uncontrolled studies or case series are the lowest. This approach has rapidly become the standard for evidence-based medicine and allows the reader to assign a level of credibility to an article's results. We are fortunate to have a relatively large number of RCTs on which to base treatment recommendations for OM, but most concern medical therapy and not surgery. Also, unfortunately, there have been relatively few randomized clinical trials that have assessed treatment of the complications and sequelae of OM. Medical treatment of chronic suppurative OM (CSOM) has been evaluated in some trials.

A useful way of organizing evidence is to classify each article as follows. *Level 1: RCT or Meta-analysis With Adequate Power and Sample Size.* Studies with positive results that are statistically significant and clinically important, and studies with negative results that have sufficient power (sample size) to ensure that a clinically important result was not missed. *Level 2: RCT or Meta-analysis With Inadequate Power or Sample Size.* Studies with positive results that are not statistically significant or clinically important, and studies with negative results that have insufficient power (sample size) to ensure that a clinically important result was not missed. *Level 3: Nonrandomized Clinical Trial or Cohort Study With Controls.* Studies in which the group receiving therapy was compared to a concurrent group that did not receive therapy, but were followed identically. *Level 4: Studies Without Control Groups and Other Evidence.* Descriptive reports such as case series or uncontrolled studies, or narrative reports such as consensus statements, panel reports, or traditional narrative review articles.

Within each evidence level below, articles are listed alphabetically by first author. The listing is not intended to be exhaustive, but includes the most clinically relevant high-quality studies published over the specified time frame. Also included are selected comments by Panel members on the assets and/or liabilities of some of the studies.

LEVEL 1 EVIDENCE: RCT OR META-ANALYSIS WITH ADEQUATE POWER AND SAMPLE SIZE

1. Barnett et al.[2] Single-blind RCT of 484 children ages 3 months to 3 years with AOM random-

ized to a single intramuscular dose of ceftriaxone sodium versus trimethoprim-sulfamethoxazole (TMP-SMZ) orally for 10 days. Clinical cure or improvement occurred at 3 days in 93% of the ceftriaxone group versus 95% of the TMP-SMZ group (p = not significant [NS]) and decreased by day 14 to 80% versus 82% (p = NS). There was no difference among groups for persistent middle ear effusion (MEE). Conversely, 8% of the ceftriaxone group had pain at the infection site for at least 3 days, and 24% had new-onset diarrhea (versus 9% of the TMP-SMZ group, p < .001). Equivalence in an RCT does not justify uncritical acceptance of an alternative AOM therapy. Issues of cost, accelerated bacterial resistance, and side effects must be considered.

2. Blumer et al.[3] Single-blind RCT of 205 children with AOM showing equivalent side effects and clinical outcomes for ceftibuten dihydrate versus cefprozil.

3. Cohen et al.[4] Double-blind RCT of 385 children under 2 years of age with AOM randomized to amoxicillin-clavulanate 3 times daily for 5 days versus 10 days. The clinical success rate at 12 to 14 days was higher (88% versus 77%) with 10 days of therapy than with 5 days of therapy (rate difference, 11%; 95% confidence interval [CI], 3%-20% p = .006). Although the difference between groups was statistically significant, an 11% rate difference still means that 9 children must receive additional treatment for a single clinical success to be achieved at 12 to 14 days. Further, the 95% CI is broad, and includes a benefit of only 3% at the lower limit, which is clinically meaningful. Multivariate analysis showed a larger impact of 10 days versus 5 days of therapy for children in day care: 87% versus 71% resolution (eg, a 16% treatment benefit).

4. Del Mar et al.[5] Meta-analysis of 6 RCTs of antibiotic versus placebo for initial therapy of AOM. At 24 hours, 60% of placebo-treated children were pain-free, and the rate rose to 86% at 2 to 7 days. Antibiotics did not influence pain relief at 24 hours, but reduced the risk of pain at 2 to 7 days by 41% (95% CI, 14%-60%), and the risk of contralateral AOM by 43% (95% CI, 9%-64%). The absolute benefit attributable to therapy was less impressive, suggesting that 17 children must receive early antibiotics to prevent 1 child from experiencing pain at 2 to 7 days. Further, antibiotics nearly doubled the incidence of vomiting, diarrhea, or rashes (odds ratio, 1.97; 95% CI, 1.19%-3.25%) and did not influence subsequent rates of AOM relapse or MEE resolution. Problems in generalizing these results exist, because diagnostic certainty was not always optimal, and some studies excluded children who were young (less than 2 years of age) or who had severe symptoms. Young, sick children with appropriately diagnosed AOM may derive greater antibiotic benefit (as suggested by Kaleida et al[6]) and may be more prone to suppurative complications if untreated.

5. Gooch et al.[7] Single-blind RCT of 477 children with AOM showing equivalent clinical and bacteriologic outcomes for cefuroxime versus amoxicillin-clavulanate. About one third fewer children had diarrhea with cefuroxime.

6. Gooch et al.[8] Single-blind RCT of 313 children with AOM showing equivalent clinical outcomes for cefixime versus amoxicillin-clavulanate. Diarrhea and vomiting were about one half as frequent with cefixime.

7. Khurana.[9] RCT of 263 children in school or day care with AOM randomized to azithromycin dihydrate 4 times daily for 5 days versus amoxicillin-clavulanate 3 times daily for 10 days. Comparable clinical efficacy was achieved (92% versus 90% clinical response at 14 days), with fewer treatment-related adverse effects with azithromycin.

8. Hoberman et al.[10] RCT of 868 children ages 2 months to 12 years with AOM randomized to twice-daily therapy for 10 days, twice-daily therapy for 5 days, or 3-times-daily therapy for 10 days. Clinically, efficacy was comparable for the 2- and 3-times-daily groups, but young children (age <2 years) did about 20% better with 10 days of twice-daily therapy than with 5 days of twice-daily therapy. The incidence of diarrhea was much lower in the twice-daily groups (9% to 10%) than in the thrice-daily group (27%; p < .0001).

9. Kafetzis et al.[11] RCT of 560 children with AOM randomized to 5 days versus 10 days of cefprozil therapy. Clinical efficacy was achieved in 87% after 5 days of therapy, and in 91% after 10 days of therapy; a trend was observed for poorer outcomes with 5 days of therapy in a subset of 19 children with 3 or more previous AOM episodes.

10. Kozyrskyj et al.[12] Meta-analysis of 32 RCTs of short-course (less than 7 days) versus long-course (7 days or longer) antibiotic therapy for AOM. Long-course therapy produced an 8% absolute increase in clinical resolution at 8 to 19 days (95% CI, 4% to 12%), but offered no benefit at 20 to 30 days. The AOM relapse and recurrence rates were similar for 3 months after long- versus short-course therapy. The 8% initial benefit begs the issue of clinical importance, since about 12 children would require long-course therapy to add 1 cure at 8 to 19 days. Although subgroup analysis was not possible, recent data suggest that short-course therapy may not be best for

young children. Hoberman et al[10] (see above) reported about a 20% absolute decrease in clinical success rates at 12 to 14 days and at 32 to 38 days when children younger than 2 years of age received 5 days, as opposed to 10 days, of amoxicillin-clavulanate. Cohen et al[4] (see above) had similar results.

11. MacLoughlin et al.[13] RCT of 167 children ages 1 month to 11 years with AOM randomized to cefpodoxime proxetil twice daily versus cefaclor 3 times daily for 10 days. Comparable clinical success rates occurred (94% versus 92% at 10 days), and the rates of side effects were similar.

12. Mandel et al.[14] Double-blind RCT of 267 children ages 7 months to 12 years with AOM randomized to amoxicillin for 10 days versus amoxicillin for 10 days plus amoxicillin-clavulanate for an additional 10 days. More children were effusion-free at day 20 if given the longer course of therapy, but the advantage was transient; after 90 days, comparable rates of AOM relapse and MEE prevalence were observed. The authors did not recommend extending the duration of antibiotic therapy if the child responded clinically to the initial course.

13. Mandel et al.[15] Double-blind RCT of 111 effusion-free children ages 7 months to 12 years with histories of chronic or recurrent MEE randomized to amoxicillin prophylaxis versus placebo. Amoxicillin significantly lowered the rates of MEE (33% versus 20% prevalence), AOM (0.28 versus 1.04 episodes per child-year), and OME (1.53 versus 2.15 episodes per child-year). Although statistically significant, the absolute level of clinical benefit is modest: placing a child on continuous prophylaxis for 3 months would prevent only 0.25 episodes of AOM and 0.21 episodes of OME. Whether this level of benefit would exceed the risk of accelerated bacterial resistance must be determined on an individualized basis.

14. Mandel et al.[16] RCT of 210 children with OME randomized to ceftibuten 4 times daily or amoxicillin 3 times daily for 14 days. Comparable rates of OME resolution at 2 weeks were seen by otoscopy (30% versus 27%) or algorithm (23% versus 20%) in both groups. However, by 16 weeks, about 60% of children who had been effusion-free at 2 weeks had relapsed.

15. McLinn.[17] Double-blind RCT of 677 children with AOM randomized to azithromycin 4 times daily for 5 days versus amoxicillin-clavulanate 3 times daily for 10 days. The rates of clinical cure or improvement at 11 days were comparable (88% for both), but the rate of gastrointestinal side effects was lower with azithromycin (9% versus 31%, $p < .0001$).

16. McLinn et al.[18] Single-blind RCT of 222 children with AOM randomized to ceftibuten versus amoxicillin-clavulanate. Comparable outcomes occurred with each drug.

17. Principi.[19] RCT of 484 children ages 6 months to 12 years with AOM randomized to azithromycin for 3 days versus amoxicillin-clavulanate 3 times daily for 10 days. Comparable levels of clinical success were achieved (93% versus 94%) with no difference in side effects.

18. Roark and Berman.[20] Double-blind RCT of 194 children aged 3 months to 6 years with 3 documented cases of AOM in the previous 6 months, randomized to amoxicillin prophylaxis 4 times daily, amoxicillin prophylaxis twice daily, or placebo up to 90 days. The placebo group had 2.6 cases of AOM per child-year, versus 2.8 in the 4-times-daily amoxicillin group and 3.2 in the twice-daily amoxicillin group (differences were not significant). The proportions of subjects remaining otitis-free were 63% for placebo, 64% for amoxicillin 4 times daily, and 61% for amoxicillin twice daily. The authors concluded that routine use of amoxicillin prophylaxis should be discouraged.

19. Rodriguez.[21] RCT of 259 children ages 6 months to 13 years with AOM randomized to azithromycin 4 times daily for 3 days versus cefaclor 3 times daily for 10 days. Comparable rates of cure or improvement (98% versus 97%) were seen at days 11 to 15. The cure rate at 1 month was better in the azithromycin group (97% versus 86%, $p = .033$), but only 68 of the original 259 subjects were available for late follow-up, so the result is unreliable and imprecise.

20. Scholz and Noack.[22] Double-blind RCT of 302 children with AOM randomized to erythromycin estolate or amoxicillin twice daily for 10 days. Comparable rates of clinical success (94% versus 96%) and recurrence (6% versus 5%) were seen in both groups. Bilateral AOM was a risk factor for poorer outcome; age and otorrhea were unrelated.

21. Thomsen et al.[23] RCT of 360 children ages 1 to 10 years with OME of at least 3 months' duration randomized to therapeutic levels of penicillin V versus amoxicillin-clavulanate for 28 days. Successful outcomes by tympanometry significantly favored the amoxicillin-clavulanate group at 14 days (31% versus 23%) and at 28 days (44% versus 19%). This modest level of benefit implies that between 4 and 12 children must receive amoxicillin-clavulanate to achieve 1 additional successful outcome. It also does not address the issue of whether treatment is justified at all, given the marginal benefits over placebo

in blinded RCTs (about 14%) and the short-term nature of the efficacy achieved.

22. Van Balen et al.[24] RCT of 149 children ages 6 months to 6 years with prospectively documented bilateral OME for 3 months or longer who were randomized to amoxicillin-clavulanate versus placebo for 14 days. Tympanometric resolution of bilateral OME occurred in 16% of placebo controls versus 47% of those receiving active therapy (absolute difference of 31%; 95% CI, 11%-58%; p = .001). The moderate benefit of therapy (need to treat 3 children to improve 1) must be balanced against gastrointestinal side effects (twice as common with active drug), the risk of inducing bacterial resistance, and the frequent tendency of OME to relapse.

23. Varsano et al.[25] RCT of 215 children ages 4 months to 6 years with AOM randomized to intramuscular ceftriaxone for 1 or 2 days versus amoxicillin-clavulanate 3 times daily for 10 days. Clinical success of 95% was achieved in both groups, with no differences in relapse or MEE resolution. The authors recommended ceftriaxone for AOM when children are unable to tolerate or absorb oral drugs or when anticipated compliance with oral therapy is suboptimal.

LEVEL 2 EVIDENCE: RCT OR META-ANALYSIS WITH INADEQUATE POWER OR SAMPLE SIZE

1. Altman et al.[26] Double-blind RCT of 208 patients receiving tympanostomy tubes. All received antibiotic ear drops at surgery, but some were randomized to also receive phenylephrine ear drops. Follow-up was available on 75% of the original sample. At the follow-up visit, the tubes were patent in 97.7% of phenylephrine-treated ears versus 91.4% of control ears (p = .02). The clinical relevance of this is difficult to judge, since the modest sample size makes the observed 6% difference between groups consistent with a benefit ranging from 1% to 11% (ie, the 95% CI). Analysis by ears and not patients also weakens the impact. Given the potential serious (albeit rare) adverse effects of phenylephrine in children, combined with the small and imprecise benefit seen in this study, it is hard to concur with the authors' conclusion that "use of phenylephrine following TT insertion considerably reduces the incidence of TT obstruction."[26(p1236)]

2. Bertin et al.[27] Double-blind RCT of 219 children ages 1 to 6 years with cefaclor-treated AOM randomized to ibuprofen, acetaminophen, or placebo for 2 days. At day 2, the levels of tympanic membrane inflammation were comparable among groups, but the ibuprofen group had better pain relief (93%) than the placebo group (75%; p < .01); the acetaminophen outcome (90% pain relief) was statistically comparable to the placebo outcome. The sample size was not large enough to estimate the extent of the effect with sufficient precision to ensure that the results were clinically meaningful. If we accept the 18% boost in pain relief achieved with ibuprofen at face value, then nearly 6 children need treatment to relieve pain in 1 child at 2 days.

3. Blumer et al.[28] Single-blind RCT of 154 children with AOM randomized to ceftibuten 4 times daily or cefaclor 3 times daily for 10 days. Clinical success was seen in 89% of children receiving ceftibuten versus 88% receiving cefaclor. Although suggesting equivalence, the modest sample size is consistent with a 95% CI favoring ceftibuten up to 12% or cefaclor up to 10%.

4. Cannon.[29] RCT of gentamicin sulfate drops versus control for prevention of early post–tympanostomy tube otorrhea in 100 children. Otorrhea was observed in 14% of the gentamicin group, but in only 8% of controls (not significant). The study has low power, but suggests it is extremely unlikely that gentamicin drops confer any benefit.

5. Daly et al.[30] Double-blind RCT of 80 children ages 6 months to 8 years randomized to TMP-SMZ plus oral prednisone versus placebo for 14 days after surgery, and followed at 3-month intervals for 12 months. The regimen of TMP-SMZ plus oral prednisone significantly reduced tube obstruction or extrusion within 3 months (4% versus 17% for placebo; p = .01), but the rates of repeat intubation, otorrhea, and recurrence of OM were similar. Larger studies are needed to determine whether the short-term benefit observed is clinically relevant.

6. Goldblatt et al.[31] RCT showing equivalent results of ofloxacin ear drops versus oral amoxicillin-clavulanate therapy for tube-related otorrhea. The implication is that many children could avoid the need for oral therapy by using drops alone. (Also see below, Intratemporal (Extracranial) Complications and Sequelae.)

7. Hemlin et al.[32] Double-blind RCT of 142 children ages 2 to 12 years randomized to 10 days of cefixime plus betamethasone versus cefixime alone versus placebo for unilateral or bilateral OME lasting 3 months or longer. After 2 weeks, the rates of improvement (resolution of OME in at least 1 ear) were 44% with antibiotic and steroid, 20% with antibiotic alone, and 5% with placebo (p < .005). The relapse rates were high and comparable among groups, with only 11% improved at 6 weeks and 10% at 6 months. Although the 24% initial boost in resolution from the steroid appears promising, this probably overstates the effect, since outcomes were only

given for improvement, not for complete resolution of OME in all ears. Even so, it still means more than 4 children needed to be treated to achieve a short-term benefit in 1 child.

8. Hoberman et al.[33] Double-blind RCT of 54 children ages 5 to 19 years with otalgia and AOM randomized to Auralgan or olive oil drops. After 30 minutes, a 25% reduction in pain occurred in 96% of Auralgan subjects versus 70% of controls ($p = .02$); no significant differences were noted between groups for an outcome of 50% pain reduction. It is unclear whether the small benefit of Auralgan seen in this study (clinically important?) can be generalized to children younger than 5 years of age.

9. Pearson et al.[34] RCT of topical antibiotic ear drops versus no drops for 5 days after tympanostomy tube insertion. No benefit was found at 3 months with regard to tube blockage, tube extrusion, or degree of audiometric improvement.

10. Pignataro et al.[35] Meta-analysis of 6 studies of mucoactive therapy with *S*-carboxymethylcysteine versus placebo for OME in children ages 3 to 12 years. Four studies had tympanometric outcomes, yielding an odds ratio of 2.25 favoring active therapy, but the 95% CI was not significant (0.97%-5.22%). If data are used from all 6 studies with a more subjective outcome measure of "overall clinical improvement," the odds ratio (2.31) achieves statistical significance (95% CI, 1.28%-4.20%). The results from this meta-analysis are questionable, because the individual studies were small, inconsistent, and of varying quality. Moreover, the outcome criteria were based on improvement in 1 or both ears, ie, partial resolution, which produces resolution rates higher than those produced when clearance in all affected ears is required (complete resolution).

11. Reidpath et al.[36] Meta-analysis of 6 studies (5 published and 1 unpublished), yielding an odds ratio for improvement with autoinflation of 1.85 (95% CI, 1.22%-2.80%). The validity of this pooled estimate is highly suspect, since all studies were short-term, unblinded, and heterogeneous. Although autoinflation is promising, the authors concluded that it cannot be recommended until benefits are replicated in a large, well-designed RCT.

12. Shinkwin et al.[37] RCT of gentamicin-hydrocortisone drops versus saline control for prevention of early postoperative tympanostomy tube otorrhea in 161 subjects. Otorrhea was observed in 9% of controls and 1% of the active-therapy group ($p < .005$), but the issue of clinical importance versus risk of therapy remains.

13. Turik and Johns.[38] Single-blind RCT of 205 children with persistent AOM after amoxicillin therapy randomized to cefaclor or cefuroxime axetil twice daily for 10 days. Comparable levels of clinical success occurred at 11 to 16 days (92% versus 89%), but the 95% CI was consistent with up to an 11% advantage for cefaclor. No difference was observed at 20 to 26 days. The cefaclor group had less diarrhea. These 2 drugs achieved similar outcomes, even though the in vitro activity of cefuroxime was superior to that of cefaclor for the bacterial pathogens responsible for AOM. This study suggested that AOM symptoms persisting after initial antibiotics were more likely to be inflammatory than infectious, and that natural history (not drug spectrum) accounted for most of the clinical resolution observed.

14. Turner and Darden.[39] Double-blind RCT of 44 infants 6 to 18 months of age with viral upper respiratory tract infections and abnormal middle ear pressure (≤ -100 mm H_2O) randomized to intranasal phenylephrine drops versus placebo. Middle ear pressure remained abnormal after treatment for 88% of the phenylephrine group versus 76% of controls. It was concluded that topical nasal therapy does not improve abnormal middle ear pressures during the common cold.

15. Uhari et al.[40] Double-blind RCT of 306 day-care children randomized to xylitol chewing gum versus sucrose control for 2 months. There was an absolute decrease in AOM occurrence of 9% attributable to xylitol ($p = .04$). On the basis of these results, a child would need to consume 4,100 pieces of gum over a 1.1-year period to prevent a single AOM episode, so the clinical relevance of this mode of prevention is of uncertain importance.

16. Uhari et al.[41] Double-blind RCT of 857 day-care children randomized to xylitol syrup, gum, or lozenges versus placebo for 3 months. On a relative basis, xylitol decreased the occurrence of AOM by 30% for syrup ($p = .006$), 40% for chewing gum ($p = .012$), and 20% for lozenges ($p = .180$). On an absolute basis, the results are less impressive: xylitol would have to be used between 1.0 and 2.8 years to prevent a single AOM episode. Further, the amount of xylitol needed to be consumed to prevent a single AOM episode is about 1,800 teaspoons of syrup, 5,600 pieces of gum, or 15,000 lozenges, so, again, there are concerns about the utility of this mode of prophylaxis.

LEVEL 3 EVIDENCE: NONRANDOMIZED CLINICAL TRIAL OR COHORT STUDY WITH CONTROLS

1. Brook and Gober.[42] Prospective nonrandomized study of oropharyngeal bacteria in 40 children followed for 9 months while receiving antimicrobial prophylaxis for recurrent AOM. At baseline, 20% of

the amoxicillin group had β-lactamase–producing bacteria, rising to 100% at 5 months, then decreasing to 15% 2 to 3 months after prophylaxis was discontinued. Sulfisoxazole acetyl did not alter bacterial resistance rates.

2. Dingle et al.[43] Fifty children had a mini–titanium grommet inserted in 1 ear and a mini–Teflon grommet inserted in the other. The incidence of tympanosclerosis was similar in each ear, suggesting that the mass of a grommet has a lesser role than previously suggested in the pathogenesis of tube-related tympanosclerosis.

3. Hampton and Adams.[44] Prospective study of 121 children with 1 tympanostomy tube inserted anteriorly in the tympanic membrane and the other inserted posteriorly. The perforation rate was 2.8%, and showed a nonsignificant trend toward being higher for anteriorly placed tubes (3.7%) than for posteriorly placed tubes (1.8%).

4. Jamal.[45] Prospective nonrandomized study of 72 patients, 32 of whom received xylometazoline hydrochloride nasal drops in the ear in the postoperative period. After 3 months, tube obstruction occurred in 11% of the control group and none of the drug group ($p = .088$).

5. Postma et al.[46] Prospective study of 456 consecutive cases plus 162 controls from a well-baby examination group. Children in day care versus home care had higher rates of initial tube insertion (31% versus 11%; $p < .0001$) and reintubation (36% versus 11%; $p < .0001$). Young age was also a significant risk factor.

6. Rosenfeld.[47] Prospective nonrandomized study of 122 children with bilateral OME for at least 3 months or unilateral OME for at least 6 months treated with antibiotic plus prednisolone for 10 days versus observation alone. Resolution of OME in all affected ears occurred at 21 to 28 days in 32% of actively treated children versus 2% of controls ($p < .001$), but the difference was no longer significant at 6 months (25% overall resolution rate). The level of evidence here is weak, considering that several other RCTs exist, but supports the conclusion of a short-term benefit for some.

7. Salata and Derkay.[48] Prospective nonrandomized study of water-related otorrhea in 533 children with tympanostomy tubes in 4 groups: antibiotic ear drops, prefabricated ear molds, swimmers without water precautions, and nonswimmers. Most otorrhea was upper respiratory tract infection–related, and was not significantly influenced by presence or absence of water precautions. Otorrhea occurred in 32% of swimmers (44 of 138) and 35% of nonswimmers (41 of 116).

8. Strachan et al.[49] Historical cohort study of 51 children who received tympanostomy tubes and 53 age-matched controls who received Shepard grommet tubes examined a minimum of 6 years after surgery. Perforations occurred in 24% of children in the tympanostomy tube group versus 2% of children in the grommet group ($p = .001$), and the rate was proportional to the duration of intubation. In contrast, the rates of hearing loss, retraction, and tympanosclerosis were similar for both groups.

LEVEL 4 EVIDENCE: STUDIES WITHOUT CONTROL GROUPS AND OTHER EVIDENCE

1. Bauchner et al.[50] In a spin-off of the intramuscular ceftriaxone versus oral TMP-SMZ study noted above (level 1 evidence, Barnett et al[2]), 83% of 327 parents whose children had single-dose intramuscular therapy for AOM said they would prefer such therapy again in the future. Parental choice should be considered for regimens of equal efficacy, but physicians need to inform parents about assets and liabilities of the various options available for treatment of AOM.

2. Bergus and Lofgren.[51] The authors developed a decision analysis model based on probabilities obtained from the literature and outcome disutilities obtained by interviewing 37 parents. Influencing factors included the number of anticipated AOM episodes in the coming year, the percentage of episodes anticipated to be severe, and how parents rated tympanostomy tubes compared with antibiotics. Tympanostomy tubes were deemed preferable to prophylactic antibiotics in the base model, but results varied on the basis of the character of the infections and the values parents gave to potential outcomes.

3. Berman et al.[52] Using a Medicaid database, the authors found that 88% of over 12,000 AOM episodes were successfully treated with a single antibiotic, regardless of the cost or antimicrobial spectrum of the initial drug. Although based on data not originally collected for research, these results support the concept that most AOM resolves regardless of proposed therapy or compliance with it, and that the nuances of initial antibiotic selection have a minimal effect on clinical outcomes.

4. DiFranza and Lew.[53] Systematic literature review suggests that passive smoking causes 354,000 to 2.2 million annual episodes of OM in children in the United States, and 5,200 to 165,000 tympanostomy tube insertions. The broad ranges suggest large uncertainty, and it remains to be shown that cessation of passive smoking prevents OM.

5. Dowell et al.[54] Representatives of the Centers

for Disease Control and Prevention and the American Academy of Pediatrics proposed 6 key principles for management of OM. 1) Episodes should be classified as AOM or OME. 2) Antimicrobials are indicated for AOM; however, diagnosis requires documented MEE and signs and symptoms of acute local or systemic illness. 3) Uncomplicated AOM may be treated with a 5- to 7-day course of antimicrobials in certain patients. 4) Antimicrobials are not indicated for initial treatment of OME; treatment may be indicated if effusions persist for 3 months or more. 5) Persistent MEE after therapy for AOM is expected and does not require retreatment. 6) Antimicrobial prophylaxis should be reserved for control of recurrent AOM, defined by 3 or more distinct and well-documented episodes in 6 months or more than 4 episodes in 12 months. One could argue with the finer points of some of these recommendations, but for the most part they are reasonable.

6. Dowell et al.[55] A panel of experts from the United States and abroad provided a consensus report on treatment regimens for AOM recognizing concerns for multi–drug-resistant *Streptococcus pneumoniae*. The report recommended considering increasing the dose of amoxicillin from 40 mg/kg per day to 80 to 90 mg/kg per day as initial empiric therapy for AOM. The regimens suggested for patients with clinically defined treatment failure after 3 days of therapy were amoxicillin-clavulanate, cefuroxime axetil, and intramuscular ceftriaxone (3 daily doses). The recommendations are based primarily on bacteriologic data and are not supported by clinical evidence demonstrating improved outcomes with the treatment recommendations.

7. Force et al.[56] Feasibility study of 10 children ages 3 to 8 years with tympanostomy tubes and *Pseudomonas aeruginosa* otorrhea unresponsive to oral antibiotics. By day 7, 90% were cured or improved.

8. Friese et al.[57] In this prospective observational study, 103 children were treated with a variety of homeopathic treatments and another group of 28 patients received nasal drops and antibiotics. Both groups improved.

9. Goldstein et al.[58] Follow-up study of 391 tubes in 165 patients: 1% had persistent perforations, 11% had tympanosclerosis, and 20% had otorrhea. Limited follow-up on many of the patients.

10. Hellier et al.[59] Survey study of parental opinion after tympanostomy tube insertion showing 92% improved hearing, 74% reduced frequency of ear infections, 87% reduced frequency of doctor visits, and 71% less missed time from school. The survey did not have psychometric validity, and the technique of asking people to directly provide a retrospective estimate of perceived change is bias-prone (eg, highly influenced by the state of the child at the time the question is asked).

11. McCarty.[60] Noncontrolled clinical trial of 210 children with AOM treated with azithromycin for 5 days. Bacteriologic and clinical efficacies were comparable to those of existing antimicrobial regimens. Side effects were seen in 9%.

12. Pichichero et al.[61] Noncontrolled, open-label, multicenter trial of 262 children ages 6 months to 12 years with recurrent AOM, persistent AOM, or multiple prior episodes of AOM, treated with cefprozil twice a day for 10 days. Clinical success rates ranged from 75% to 93% of the children and varied by causative pathogen. Pathogens were similar to those found in recently untreated AOM. Older children had clinical success more frequently than younger children ($p < .001$).

13. Roark et al.[62] Survey study of OM management strategies chosen by pediatricians versus family physicians for hypothetical case scenarios. Family physicians were significantly more likely to prescribe high-cost antibiotics, treat persistent MEEs, administer oral decongestants, and refer for tympanostomy tube insertion. These wide practice variations are interesting, but relate to hypothetical treatment scenarios presented to a limited group of Colorado physicians.

14. Rosenfeld et al.[63] Prospective cohort study of 186 children ages 6 months to 12 years with chronic or recurrent OM in which a disease-specific quality-of-life measure, the "OM-6," was developed and validated. For a subset of 37 children surveyed before and after tympanostomy tube insertion, 95% had improved quality of life (57% had a large improvement) and 5% experienced trivial changes. The data are considered preliminary.

15. Saito et al.[64] Showed a 4% to 14% perforation rate for tympanostomy tubes (42 ears) and a 13% rate for Paparella type II tubes (110 ears), proportional to the intubation period. Paper-patch tympanoplasty successfully closed 97% of perforations when performed at the time of tube removal.

INTRATEMPORAL (EXTRACRANIAL) COMPLICATIONS AND SEQUELAE

Between 1995 and 1999, there were many reports in the literature related to the complications and sequelae of OM. For this review, we have excluded cholesteatoma, since this disease is the subject of an ongoing series of international symposia.

Hearing Loss. There were several reports related to hearing loss, both conductive and sensorineural, associated with MEE. Both Ellis et al[65] and Hunter et al[66] described the conductive hearing impairment, and Gravel et al[67] and Rahko et al[68] showed that the loss is reversible with the resolution of the MEE.

Seely et al,[69] from Sierra Leone, evaluated the hearing in children who had a chronic perforation of the tympanic membrane with or without suppuration, and found the loss was greater than that found with only MEE; of the 37 ears that had dry perforations, 33 (89%) had a pure tone average of 26 dB or greater, and of the 100 ears that had CSOM, 96 (96%) also had this degree of hearing loss.

Mutlu et al[70] reviewed the audiograms of 71 children (119 ears) who had OME and found that 9% had either temporary sensorineural involvement (temporary threshold shift) that improved when the effusion resolved or possibly permanent sensorineural hearing loss, which they attributed to the effusion. Also, ultrahigh-frequency hearing (9,000 to 20,000 Hz) was reported to be affected in children with OM; it would not be detected in routine audiometric testing[71] and may be affected for the long term.[72] Blakley and Kim[73] reported that CSOM, cholesteatoma, or both can be associated with sensorineural hearing loss.

Sone et al,[74] in a study in animals, reported that sensorineural hearing loss associated with pneumococcal OM may be related to expression of intercellular adhesion molecule–1. In other animal studies, it has been shown that OM can influence the integrity of the inner ear through both inflammatory mediators[75,76] and bacterial products.[77-80] Histopathologic findings and clinical history have underlined the possible role of labyrinthine fistula in chronic OM with inner ear consequences.[81-84] Some mediators, such as platelet activating factor and interleukin-2, have particularly been suspected to affect the outer hair cells.[85,86] In the particular case of bone remodeling occurring during OM,[87] substance P and neural control have been incriminated.[88] From a clinical point of view, it seems more and more that chronic OM is associated with sensorineural hearing loss.[89-91]

Vestibular, Balance, and Motor Dysfunctions. Studies of labyrinthine function by Casselbrant et al[92,93] in children with MEE confirmed that the vestibular system is adversely affected, and that after tympanostomy tube placement, it returns to normal. Also, tests of motor proficiency yielded abnormal results in children when MEE was present.[94-96]

Chronic Perforation of Tympanic Membrane: Tympanoplasty. The rate of tympanoplasty for repair of chronic perforation of the tympanic membrane was noted in several reports. Vartiainen and Vartiainen[97] operated on 60 children during a 15-year period and found that previous adenoidectomy had a negative effect on outcome. Caylan et al[98] reported on a similar number of children who had surgical repair of a chronic perforation in an Italian center during a 10-year period; they also reviewed the literature from 1974 to 1991 and found 640 children had tympanoplasty as reported in 12 studies. Another review of the literature, from 1979 to 1995 by Bajaj et al,[99] reported 870 patients had tympanoplasty, and still another review by Denoyelle et al[100] found 1,741 cases reported between 1985 and 1998. Vrabec et al[101] reported on their meta-analysis of tympanoplasty from 1966 to 1997 and indicated that the success rate increased with advancing age in children, and that no other parameters significantly influenced the success rate. Cueva,[102] in a review of outcomes in children and adults, reported success using areolar temporalis fascia, and O'Hare and Goebel[103] recommended anterior subannular T-tubes for long-term middle ear ventilation, placed at the time of tympanoplasty.

Chronic Suppurative Otitis Media. In a review of over 50 publications related to the epidemiology of CSOM in all age groups, Bluestone[104] reported that populations could be separated into degrees of risk: the highest risk was in Inuits of Alaska, Australian Aborigines, and certain Native Americans (eg, Apache and Navajo); moderately high rates were found in certain natives of the South Pacific islands and some African populations; relatively low rates were found in Korea, India, and Saudi Arabia; and the lowest risk was reported from the highly industrialized countries such as the United States, Denmark, the United Kingdom, and Finland. In a more recent study of 591 children from Greenland, Homoe et al[105,106] reported that CSOM was related to ethnicity and was most common when both parents were native Greenlanders.

The microbiology of CSOM continues to be reported as primarily caused by *P aeruginosa* in different parts of the world.[107-109] Stenquist et al[78] reported that this bacterium in the middle ear caused inner ear dysfunction in the rat.

Jaisinghani et al[110] reported "intratympanic membrane cholesteatoma" in 5 of 243 human temporal bones with chronic OM. The fact that in all cases, this type of cholesteatoma was in the pars tensa suggested that cholesteatoma is one of the sequelae of chronic OM. In another report from the same laboratory, based on examination of 150 temporal bones from 97 subjects with chronic OM, Jaisinghani et al[111] reported a significant correlation between tympanic membrane and middle ear pathology, which included myringosclerosis with granulation tissue, myringosclerosis with ossicular pathology, retraction pocket with cholesterol granuloma, retraction pocket with cholesteatoma, retraction pocket with ossicular pathology, perforation with ossicular pathology, and hemorrhage with granulation tissue. But almost half of the bones with middle ear pathology had no associated tympanic membrane pathology. Jang and Merchant[81] reported labyrinthine fistulae with chronic OM in 7 of 115 human temporal bones. They suggested that the fistula can be caused by cholesteatoma, by granulomatous chronic OM without cholesteatoma, or even by localized infection. They also reported that in most cases, reactive inner ear changes do not occur at the fistula site. However, if overwhelming infection or surgical trauma breaches the natural barriers of the endosteum and membranous labyrinth, the fistula may allow rapid dissemination of infection throughout the inner ear.

Reports related to the medical treatment of CSOM primarily addressed the continued concern about ototoxicity of ototopical agents that are in common usage but not approved by the US Food and Drug Administration (FDA) for CSOM[112,113]; new ototopical agents such as ofloxacin, which was shown to be both relatively safe and effective and is approved by the FDA[31,114-118]; and topical ciprofloxacin with hydrocortisone,[119-122] which also appears not to be associated with ototoxicity.[123,124] Thorp et al[125] evaluated acetic acid as compared with aluminum subacetate (Burow's solution) in vitro and found both to be effective. Dohar et al[126,127] found that oral agents currently approved for OM in children were ineffective in vitro for treatment when *Pseudomonas* was the causative organism; however, 40% of strains were susceptible to TMP-SMZ. Smith et al[128] reported that "dry mopping," a common treatment in Kenya, was ineffective as compared with oral antibiotics and ototopical agents. Vartiainen et al[108] reported that intensive medical treatment should precede recommending major ear surgery, since the outcome of surgery is not as favorable if medical treatment is withheld.

Mastoiditis. Acute mastoiditis is still a prevalent complication of OM. In a review from 1980 to 1995 from the Children's Hospital of Pittsburgh by Goldstein et al,[129] 72 infants and children were treated for acute mastoiditis, but only 18 (25%) required mastoidectomy; the remaining 54 (75%) had only medical treatment (with and without myringotomy). A similar review of 58 Australian children from 1982 to 1993 by Harley et al[130] found similar outcomes: 13 (22%) had mastoidectomy, whereas 45 (78%) did not. But another center reported that most patients required a mastoidectomy.[131] Subperiosteal abscess was present in all patients in 1 study,[132] in two thirds of children in another,[133] and in only one third in yet another study.[129]

Antonelli et al[134] found that erosion of the cortical plate overlying the sigmoid sinus on computed tomography scan was a helpful finding in distinguishing between osteitis and nonosteitis acute mastoiditis. Microbiology of the infection was reported to show *S pneumoniae*, *Streptococcus pyogenes*, or *Haemophilus influenzae*, but there may be an unusual organism, such as *P aeruginosa*, if there has been otorrhea.[129,130,133] Also, other unusual organisms were reported, such as *Mycobacterium avium*[135] and *Mycobacterium chelonae*.[136]

Facial Paralysis. Facial paralysis was reported to be found not only associated with acute OM[137,138] (even in patients with a sterile middle ear culture[139]), but also as a complication of OME.[139] Bilateral facial paralysis as a complication of AOM was reported in a child by Smith and Traquina,[140] and Fukuda et al[141] reported another child with this bilateral complication. It has also occurred as a complication of acute mastoiditis,[129,142] as a complication of leukemia,[143] and as the initial symptom of Wegener's granulomatosis.[144] Elliott et al[145] found that of 10 patients in whom facial paralysis developed as a complication of AOM, 8 had incomplete paralysis that resolved with myringotomy and intravenous antibiotics, whereas the remaining 2 had complete paralysis and required a mastoidectomy.

Atelectasis of Middle Ear and Adhesive Otitis Media. Several articles published from 1995 through 1999 can be graded by means of the evidence-based system described above.

LEVEL 1 EVIDENCE: RCT OR META-ANALYSIS WITH ADEQUATE POWER AND SAMPLE SIZE

1. Maw and Bawden.[146] In an RCT of 222 children with OME treated in 1 ear with a tympanostomy tube, atelectasis developed in 17% of untreated ears and in 15% of those treated with tubes. Very few cases (1.5% and 2%) developed severe atelectasis.

LEVEL 2 EVIDENCE: RCT OR META-ANALYSIS WITH INADEQUATE POWER AND SAMPLE SIZE

1. Hozawa et al.[147] The outcomes of several surgical techniques for adhesive OM were compared. Significant improvement of postoperative hearing was obtained after tympanoplasty with tympanostomy tube insertion. The technique of tube insertion for total adhesion cases was also described.

2. Mattsson et al.[148] Using a null mutant mouse lacking extracellular superoxide dismutase, they tested the hypothesis that increased production of free radicals can cause myringosclerosis. Null mutant mice and wild-type, control mice were myringoto-

mized and kept in ambient air for 3 weeks. Myringosclerosis developed in the pars flaccida in both groups, but not in the pars tensa. No difference concerning the extent or number of sclerotic lesions could be distinguished between the null mutant and the wild-type mice.

LEVEL 3 EVIDENCE: NON-RANDOMIZED CLINICAL TRIAL OR COHORT STUDY WITH CONTROLS

1. Giebink et al.[149] In a cohort study of 157 children, the proportion of tympanic membranes with atrophy, tympanosclerosis, and pars tensa retraction increased significantly between 3 and 7 years after enrollment. The tympanic membrane pathology progressively increased in many children for at least 7 years after tube treatment of chronic OME.

2. Narula.[150] Thousands of children with persistent OME were analyzed to evaluate the clinical significance of the degree of pars tensa retraction. The degree of pars tensa retraction did not contribute significantly to the prediction of resolution of OME, nor to the severity of hearing impairment.

LEVEL 4 EVIDENCE: STUDIES WITHOUT CONTROL GROUPS AND OTHER EVIDENCE

1. Huang et al.[151] To understand the histopathologic changes of adhesive AOM in humans, they reviewed temporal bone sections. In the 338 pairs of temporal bone specimens, 20 cases (26 ears) showed characteristics of adhesive AOM. According to the main pathological characteristics, the histopathologic changes of the 26 ears were staged as exudation, inflammatory granulation formation, histocyte response, and collagen proliferation.

2. Huang et al.[152] This paper analyzed 110 cases (120 ears) of adhesive AOM collected during the 25 years from 1967 to 1992. Clinical observation found that the lesions of adhesive AOM affected all parts of the middle ear, even the round and oval windows. After the tympanic adhesions were surgically separated and the lesions were removed, polymeric silicone film or a slice of homologous cartilage was put into the tympanum to prevent a new adhesion. However, hearing did not recover to an available level.

3. Goksu et al.[153] To evaluate the clinical importance of the petrosquamosal lamina (Korner's septum), a retrospective review of 688 mastoidectomies was performed. The prevalence of Korner's septum was 30.4% among ears with retraction pockets or retraction and/or adhesion of the tympanic membrane, 6.58% in normal ears, and 17.4% in ears with chronic OM without retraction pockets or retraction and/or adhesion of the tympanic membrane.

4. Miura et al.[154] The authors examined the mastoid pneumatization of habitual sniffers by using computed tomography, and the change of negative middle ear pressure induced by sniffing by using tympanograms. In 20 ears with cholesteatoma or adhesive OM, the areas of the mastoid cavity were significantly smaller than those in 26 ears with OME or attic retraction and in 8 normal ears of habitual sniffers ($p < .01$ and $p < .0001$, respectively). By contrast, in the 4 ears with sniff-induced middle ear disease, the recovery of negative middle ear pressure in 5 minutes without swallowing was less than 10 mm H_2O, whereas in all 7 ears with normal eardrums, negative middle ear pressure recovered by more than 20 mm H_2O in 5 minutes.

5. Thomasen et al.[155] Type 3 pneumococci were inoculated into the right tympanic bullae of 25 rats, and the pathological changes of the middle ear mucosa was observed. The process of formation of adhesion was described.

6. Tsuji et al.[156] Eustachian tube function of 32 patients with adhesive OM was described and showed a variety of types, from patulous to stenotic.

7. Yamaguchi et al.[157] Case reports of patulous eustachian tube associated with atelectatic ears and cholesteatoma were presented.

8. Tos and Thomasen.[158] Human temporal bones were examined histologically and adhesion was found in 87%. The pathogenesis of adhesion formation was speculated on according to the observation.

9. Furukawa et al.[159] The ultrastructural pathology of the tympanic membrane was examined at tube insertion. Adhesive OM only developed in cases with the degeneration of collagen fibers extending to the outer radial fiber bundles.

TYMPANOSCLEROSIS AND MYRINGOSCLEROSIS

Many articles were published related to the development of tympanosclerosis and myringosclerosis, which could be graded by the level of evidence, but none are considered to be in the level 1 evidence category.

LEVEL 2 EVIDENCE: RCT OR META-ANALYSIS WITH INADEQUATE POWER AND SAMPLE SIZE

1. Mattsson et al.[160] This study elucidated possible relationships between oxygen concentration of the middle ear cavity and the development of myringosclerosis. Three groups of rats with myringotomized tympanic membranes were exposed to different oxygen concentration of 10%, 15%, and 40%, respectively, for 1 week. All hyperoxic animals had more numerous myringosclerotic lesions than did the ambient air group. By contrast, the hyperoxic animals showed less pronounced myringosclerotic lesions or

had none whatsoever.

2. Mattsson et al.[161] *Streptococcus pneumoniae* was inoculated into the left middle ear cavity in 2 groups of rats, resulting in purulent OM. Extensive myringosclerotic lesions developed in the noninfected myringotomized animals, whereas only minimal sclerotic deposits were noted in the myringotomized animals with AOM. The nonmyringotomized rats with AOM were free of sclerotic lesions, except for minor changes found in 1 animal.

3. Mattsson et al.[162] Twenty-four Sprague-Dawley rats were myringotomized bilaterally. At 3, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72, and 84 hours after the myringotomy, 2 animals at each time were examined otomicroscopically and thereafter sacrificed. Otomicroscopically, myringosclerosis was visible in the pars tensa 24 hours after myringotomy, whereas no sclerotic lesions were noted in the pars flaccida. Histologically, sclerotic lesions were present in the pars tensa and pars flaccida 9 and 12 hours, respectively, after myringotomy.

4. Forseni et al.[163] Type 3 *S pneumoniae* was inoculated into the tympanic bullae of 67 rats, and their pathological changes were examined immunohistochemically. Acute otitis media developed in all ears, and myringosclerosis developed in 20% to 30%. The immunohistochemical study demonstrated scattered macrophages in the eardrum.

5. Strachan et al.[49] Ninety children underwent tympanostomy tube insertion as a primary procedure for OME over a 3-year period. Fifty-one patients (102 ears) in the tympanostomy tube group and 53 patients (106 ears) in the Shepard grommet group were clinically reviewed, a minimum of 6 years after surgery. A perforation rate of 24% in the tympanostomy tube group, as compared with 2% in the Shepard grommet group (p = .001), is directly attributable to tympanostomy tube insertion. Furthermore, the risk of perforation was related to the duration of the tympanostomy tube in the tympanic membrane.

6. Mattsson et al.[164] Sclerotic lesions in the tympanic membrane were experimentally elicited by keeping rats with perforated tympanic membranes in an atmosphere containing roughly 40% oxygen. The animals were treated daily with a solution containing either copper-zinc–superoxide dismutase plus catalase and deferoxamine or copper sulfate plus iron chloride applied to the traumatized area. After 1 week, the extension of myringosclerotic plaques was determined otomicroscopically. The results showed that treatment with copper-zinc–superoxide dismutase plus catalase and deferoxamine inhibited or reduced the development of myringosclerosis, whereas the ears treated with copper sulfate plus iron chloride appeared unaffected.

LEVEL 3 EVIDENCE: NONRANDOMIZED CLINICAL TRIAL OR COHORT STUDY WITH CONTROLS

1. Dingle et al.[43] Fifty children with OME undergoing grommet insertion had a mini–titanium grommet inserted into 1 ear and a mini–Teflon grommet into the other ear. Postoperative follow-up performed until after extrusion of the grommets demonstrated only a small difference between the extrusion times of the 2 grommets (a significant difference of 41 days) and no difference in the degrees of tympanosclerosis seen with each grommet.

2. Stangerup et al.[165] A cohort born in 1955 was invited to a screening examination including otomicroscopy. Pars tensa pathology, including atrophy and myringosclerosis, was found in 6% of the ears in the untreated cohort and in 17% in the treated cohort. Normal eardrums were found in 91% of the ears. Despite the increased awareness of secretory otitis, as well as the increased rate of surgical treatment, the prevalence of eardrum pathology seems to be increasing.

3. Levine et al.[166] Approximately 800 children with OME were examined to evaluate the risk factors for the development of tympanosclerosis. The appearance of the tympanic membrane changed in 12 to 16 years, from bulging and thick to tympanosclerotic and retracted. Serous effusion seemed to prevent the development of tympanosclerosis.

4. Daly et al.[167] A cross-sectional study was conducted of sequelae among children, adolescents, and adults at 4 years and at 9 to 23 years after tympanostomy tube treatment. Among the 5- to 28-year old subjects, cholesteatoma (≤1%) and perforation (≤2%) were rare. Tympanosclerosis increased with age (p < .01), and OME (flat tympanograms) decreased with age (p < .01). The older cohort was more likely to have severe retractions (18% versus 4%, p = .02), hearing loss (21% versus 10%, p < .01), and severe atrophy (24% versus 0%, p < .01) than the younger cohort, but they were less likely to have flat tympanograms (2% versus 12%, p < .01).

LEVEL 4 EVIDENCE: STUDIES WITHOUT CONTROL GROUPS AND OTHER EVIDENCE

1. Stenstrom and Ingvarsson.[168] A group of 88 otitis-prone children born from 1978 to 1981 who had more than 11 episodes of AOM were examined in 1984-85. Seventy-four of the children were reexamined in 1990-92. Surgery had been performed during this period in 25 (34%), and the most common finding was the need for tympanostomy tubes. The condition of the tympanic membranes showed im-

provement for many children. In 1984-85, 31 of the children (35%) had normal tympanic membranes, as did 44 (60%) in 1990-92. Scar/tympanosclerosis was more common at the follow-up examination: 11 (13%) versus 20 (27%). In 1984-85, 1 child was found to have a central perforation, and in 1990-92, 5 children were found to have adhesive OM, CSOM, or central perforation. No cases of cholesteatoma were found in 1984-85 or in 1990-92.

2. Yabe et al.[169] Fifty-nine cases of tympanosclerosis were investigated clinically and pathologically. Calcification in the tympanic membrane was most common in the upper quadrants of the pars tensa, and never seen in the pars flaccida. Calcification in the middle ear cavity was most common around the malleus. Preoperative audiometry revealed a stiffness curve with elevated bone conduction thresholds. Microscopic examination revealed calcification in the middle ear submucosa.

3. Goldstein et al.[58] The authors performed a retrospective review to assess the incidence of complications from the insertion of tympanostomy tubes. A total of 391 tubes were placed in 165 patients. Tympanosclerosis was found in 30 ears (11.1%). No ear showed a chronic retraction pocket or cholesteatoma. The mean pure tone average prior to tube placement was 25.0 dB; with tubes in place, it was 2.44 dB; and after the last set of tubes had extruded, it was 6.97 dB.

4. Vartiainen and Vartiainen.[170] The status and hearing function of the contralateral ears in 493 patients undergoing surgery for chronic OM were studied at the final follow-up examination of the patients. In only 37% of the patients was the contralateral ear found to be normal, with atrophy of the pars tensa and tympanosclerosis being the most common abnormal findings, followed by pars tensa and attic retractions. In 18% of the patients, the contralateral ear had also undergone surgery. In only 64% of the patients, the contralateral ears had normal ($\leq$20 dB) hearing levels, and in 21%, the hearing levels were >30 dB, the hearing function of patients with cholesteatoma being worse than that of other patients with chronic OM.

5. Hunter et al.[71] Long-term effects of OM on hearing in both conventional and high-frequency regions in children were studied. A history of OM was associated with poorer high-frequency hearing, but the presence of subtle residual middle ear dysfunction was not associated with an additional effect on high-frequency hearing. Active middle ear disease significantly affected both conventional and high-frequency thresholds. The number of intubations and frequency of OM during follow-up were significantly and positively associated with poorer high-frequency thresholds. Children at greatest risk for high-frequency hearing loss were those who required multiple intubations.

6. Riley et al.[171] Eighty children who had myringotomy performed for OME in 1984 were reviewed in 1994. This had involved surgery on 158 ears. Tympanosclerosis was only found in those ears that had tympanostomy tubes inserted (and not in ears that had myringotomy only), occurring in 48 ears (31%, or 39% of those that had a tympanostomy tube inserted). There was no link between tympanosclerosis and hearing loss. There was a tendency for more extensive tympanosclerosis to occur in those ears that had more tympanostomy tube insertions.

7. Dursun et al.[172] This study was designed to evaluate the association between certain human leukocyte antigens and tympanosclerosis. The serum levels of HLA-B35 and HLA-DR3 were significantly higher in patients with tympanosclerosis.

8. Gaihede et al.[173] This study investigated the mechanical properties of ears of 69 adolescents treated with tympanostomy tubes during childhood for secretory OM. The tympanic membranes displayed various degrees of atrophy, sclerosis, and retraction of the pars flaccida. Atrophy was quantitatively related to decreasing hysteresis and increasing compliance, while myringosclerosis showed opposite effects. Treatment with tympanostomy tubes was associated with a dramatic increase of tympanic membrane pathology (66%) in comparison to untreated ears (12%), as assessed by otomicroscopy ($p < .001$).

9. Fujiyoshi et al.[174] A type II collagen autoimmune response resulted in arthritis, auricular chondritis, and tympanosclerosis in humans and animals. Type II collagen fragment CB11–specific monoclonal antibodies induced lesions in joints, earlobes, and tympanic membranes. In immunized mice, the thickness of tympanic membranes increased to 2- to 4-fold normal size. Electron micrography showed that the arrangement of collagen fibers is irregular in both radial and auricular layers, containing fibroblasts, a homogeneous material resembling low-density cholesterol crystals, and cell infiltration.

10. Mattson and Hellstrom.[175] This study was undertaken to test the ability of fenspiride hydrochloride to prevent myringosclerosis from developing during healing of the tympanic membrane. Myringotomized rats were treated with either topical applications or intraperitoneal injections of fenspiride for 12 days, after which the tympanic membranes were examined by otomicroscopy and studied histologically by light microscopy. Topically applied fenspiride was found

to inhibit the development of sclerotic lesions, whereas intraperitoneal injections were ineffective.

11. Forseni et al.[176] Sprague-Dawley rats were exposed to a type 3 pneumococcus solution into the middle ear. Groups of rats were killed at 3, 6, and 10 days after inoculation. Macrophages, T cells, and B cells presented time-dependently in the acute inflammatory response in AOM. Macrophages were the first cells to invade the tissue after AOM induction. Some cells were found in the tympanic membrane. Large, osteoclast-like cells could be seen adjacent to the bone in the submucosa. T cells and B cells were seen in the submucosa.

12. Charabi and Tos.[177] A case report of obstruction of the eustachian tube by tumor was presented. No clinical sign was noted in this case of tube insertion without infection.

13. Wielinga and Kuijpers.[178] The eustachian tube of specific pathogen–free rats was obstructed. The induction of sterile effusions always resulted in tympanosclerotic lesions. The development of tympanosclerosis was speculated to be due to the injury to the lamina propria and/or vascular disturbance caused by inward retraction of the pars tensa.

14. Attallah.[179] After an analysis of 305 children with OME, the author concluded that early otorrhea following grommet insertion is a major factor in the development of tympanosclerosis.

OSSICULAR FIXATION OR DISCONTINUITY

No articles were published from 1995 to 1999 on ossicular fixation or discontinuity.

INTRACRANIAL COMPLICATIONS

Intracranial complications of OM and mastoiditis continue to occur primarily in developing countries, but also in highly industrialized nations (albeit less frequently). From 1994 to 1999, several articles were published related to the prevalence, bacterial etiology, diagnosis, and management of these potentially life-threatening complications.

Prevalence. In a study from France, Barry et al[180] reported that between 1977 and 1995, 79 adults had otogenic intracranial complications; this report emphasizes that these complications existed not only in children and in developing countries, but in adults in a highly industrialized nation. Panda et al,[181] from India, reported that intracranial complications developed in 45 patients between 1992 and 1995, and that 24% had more than 1 intracranial complication; also, chronic OM was responsible for suppurative complications in children, even when ear drainage was of short duration. Syms et al[182] also found that many patients with suppurative complications of OM have 1 or more other intracranial complications, such as brain abscess, otitic hydrocephalus, and epidural abscess. Dill et al[183] reported that subdural empyema is a rare complication of OM today, but is not an uncommon intracranial complication of paranasal sinusitis.

Garcia et al[184] reviewed the world literature from 1960 to 1995 and found 58 children with lateral sinus thrombosis as a complication of ear disease in both developing and developed countries; 57% had middle ear and mastoid infection, and 43% had only OM. In a study from Israel, 13 children had cases of otogenic lateral sinus thrombosis during the 15-year period 1982 to 1997, of which 5 (38%) were complications of AOM, whereas the remaining 8 (62%) were due to chronic OM.[185] Richardson et al[186] reviewed 124 children in Great Britain who had meningitis, but found OM to be an uncommon cause, especially since 92 (74%) had meningococcal meningitis, and *Neisseria meningitidis* is not an otitic bacterial pathogen.

Diagnosis. Albers[187] confirmed persistent fever and headache in association as the most common early symptoms of an intracranial complication and stressed the need to make an early diagnosis to reduce morbidity and mortality. Tomkinson et al[188] and Saah et al[189] reported that magnetic resonance imaging, especially with gadolinium, provides excellent resolution of intracranial suppuration and its consequences, such as edema, thrombosis, and hydrocephalus. Magnetic resonance imaging and magnetic resonance angiography are recommended diagnostic procedures for lateral sinus thrombosis.[190] A magnetic resonance imaging study that reveals high signal intensity on T1- and T2-weighted images and absence of flow within thrombosed sinuses on gradient echo images indicates this complication.[184] Holzmann et al[191] preferred contrast-enhanced computed tomography in their review of 6 children who had lateral sinus thrombosis as a complication of OM and mastoiditis. Sennaroglu et al[192] and Tomkinson et al[188] reported that magnetic resonance imaging could be used to diagnose otitic hydrocephalus by showing thrombosis in the sigmoid and transverse sinuses.

Microbiology. In a study from Greece, anaerobic bacteria were the predominant bacterial pathogens in 21 patients with brain abscess.[193] But Grigoriadis and Gold[194] reported a case from Toronto and found 23 other case reports in the literature citing pneumococcus as the bacterial cause of brain abscess. Syms et al[182] isolated several bacteria from the ears of 6 patients with lateral sinus thrombosis, such as *Bacte-*

roides fragilis, *Peptostreptococcus* species, *Proteus* species, and *Pseudomonas* species.

Management. De Falco et al[195] reported that treatment for subdural empyema includes intensive intravenous antimicrobial therapy and neurosurgical drainage of the empyema through bur holes or craniectomy — or in infants, possibly only percutaneous aspiration. Brook[196] reported that brain abscess, especially in the early phase of cerebritis, may respond to antimicrobial therapy without surgical drainage, and emphasized the need for early diagnosis. Garcia et al[184] wrote that if lateral sinus thrombosis is only due to OM, myringotomy and tympanostomy tube insertion may be effective without the use of mastoid surgery or surgery directed at the lateral sinus.

Job et al[197] concluded from their review that if meningitis is due to CSOM or cholesteatoma or both, a tympanomastoidectomy is frequently required and should be performed when the patient's condition is stable; if there is bilateral middle ear disease and the offending side is uncertain, then bilateral tympanomastoidectomy is a reasonable procedure.

Outcome. In a review of 122 patients in Taiwan in whom brain abscess developed from several sources, the mortality rate was 3.8% when the infection was caused by an otolaryngological focus, as compared to 24% when the abscess was from other sources.[201] In South Africa, 58 children had lateral sinus thrombosis from 1990 to 1995, 3 of whom died (5%); additionally, all had other intracranial complications, such as brain abscess, sepsis, and cavernous sinus thrombosis.[184] In an article from Israel on 13 children with lateral sinus thrombosis, 1 child, who also had a brain abscess, was reported to have died.[185] One case was reported of a 6-year-old child with Mondini dysplasia who had a previously inserted cochlear implant and died of bacterial meningitis caused by an attack of OM (in the ear contralateral to the implant) that progressed to suppurative labyrinthitis and then meningitis.[199]

CONSIDERATIONS REGARDING OTITIS MEDIA CLINICAL TRIALS

In its 1995 Report, this Panel described factors critically important in conducting valid, interpretable studies of management of OM that can be applied to clinical practice.[200] The above review of the literature published during the past 4 years shows that many of these factors were included in the design, methodology, and evaluation of many, but not all, of the studies. This Panel continues to stress the need to improve the quality of research that is directed at determining the most safe and effective methods of treatment and prevention of OM.

The current Panel concluded that the considerations regarding clinical trials published in the previous Conference Report are still goals for the future. Thus, the following is essentially a restatement of these considerations, together with additional goals considered to be currently needed.

1. Clear definition of the condition(s) being studied is paramount.

2. Variables that are known or suspected to affect outcome, such as age, gender, anatomic and environmental factors, and disease laterality, should be identified and managed in the experimental design by appropriate stratification or analysis methods.

3. Entry and exclusion criteria should be clearly stated.

4. Rigorously defined end points and outcome criteria are essential.

5. Biostatistical consultation early in design development and during analysis and interpretation should be incorporated into every clinical trial to minimize the likelihood of undertaking a trial that cannot be interpreted. Sample size considerations are particularly important in this regard.

6. Methods for retaining subjects and maximizing (and documenting) treatment compliance should be incorporated into study design.

7. Assessment of case records by an independent observer to confirm maintenance of criteria is highly desirable.

8. Economic costs of treatments and benefits, in terms of both treatment efficacy and quality-of-life considerations, will become increasingly important in the era of diminishing health care resources.

9. Ethical considerations, as reviewed by Ruben,[201] are paramount in designing clinical trials; one must recognize that there are both pros[202] and cons[203] to the RCT.

10. Measures of health-related quality of life and patient satisfaction should be performed in addition to traditional measures of disease outcome.

11. Measures of clinical benefits, and not simply statistical significance, should be emphasized, to define appropriate expectations for parents and health care providers.

FUTURE RESEARCH GOALS

The current Panel also reviewed the nonsurgical and surgical goals for future research in the 1995

Conference Report and concluded that to date, relatively few had been achieved. The following is essentially a restatement of these goals.

GENERAL

1. What can we learn about the natural history of untreated AOM and OME from RCTs? Placebo and untreated control groups in these studies are a valuable but underutilized source of data on natural history. Research is needed to derive valid techniques for pooling these data, with emphasis on generalizability (external validity) of results.

2. What can we learn about the prognosis of recurrent AOM from control groups in prophylaxis trials? Again, placebo groups provide invaluable information, with the recognition that children in these studies do receive short-term therapeutic antibiotics for individual AOM episodes when they occur. Research is needed to derive valid techniques for pooling these data, with emphasis on generalizability (external validity) of results.

NONSURGICAL

1. Do all children with uncomplicated AOM require initial antibiotic therapy? Existing RCTs and experience in Scandinavian countries suggest that some may not, although it is difficult to generalize the results of RCTs, since many exclude young sick children (particularly those under 2 years of age), and the degree of diagnostic certainty is highly variable. Studies are needed to identify demographic, clinical, and inflammatory (biochemical, microbiological, and immunologic) factors that predict antimicrobial efficacy (bacteriologic and clinical), complications, and sequelae, especially in groups at high risk for poor outcome. The goal of these studies should be to develop criteria that distinguish children who can be safely initially observed rather than treated with antimicrobial agents.

2. What is the true impact of initial antimicrobial therapy on suppurative complications of AOM? Assuming that children who initially do not receive antibiotics are treated if AOM symptoms persist beyond 24 to 48 hours, is the chance of mastoiditis or meningitis increased?

3. Antimicrobial drug pharmacokinetics in the middle ear during OM should continue to be studied, including the relationship of antimicrobial pharmacokinetics to co-therapies, such as anti-inflammatory drugs. There is a need to understand why antibiotic-susceptible bacteria are not eradicated by antimicrobial drugs that have in vitro activity.

4. Emerging bacterial resistance, especially of multi–drug-resistant *S pneumoniae*, creates an imperative to design OM clinical trials with bacteriologic-dependent variables, and additional studies are needed to compare clinical and bacteriologic outcomes. Studies of new antimicrobial drugs should include middle ear effusion culture during or immediately after drug treatment. Studies not measuring bacteriologic outcomes may need to be much larger than those measuring bacteriologic outcomes. To what degree should bacteriologic studies of AOM guide clinical therapy? Can we document better outcomes with the Centers for Disease Control and Prevention and the American Academy of Pediatrics recommendations, or do similar clinical outcomes occur regardless of nuances in drug selection? Are AOM initial treatment failures primarily caused by an excessive inflammatory response, or are resistant bacteria the cause?

5. Further studies are needed to define the contribution of anti-inflammatory drug treatment (eg, steroidal, nonsteroidal, lymphokine antagonist) of OME. Since treatment with corticosteroid drugs in studies reported to date appears to have had no effect or only transient effects on OME, studies of anti-inflammatory treatment earlier in the disease course (eg, with antibiotics during treatment of AOM) are indicated, especially in children at high risk for development of OME. These treatment approaches should be investigated in relation to other medical and surgical treatment modalities, and should be designed with appropriate controls. Examinations should be timed so as to adequately measure both short- and long-term outcomes.

6. Studies are needed to determine the indications for and the nature of immunologic evaluation of children with recurrent AOM. Ultimately, these studies should determine whether immunologic dysfunction or lack of acquired immunity contributes to the pathogenesis of recurrent AOM or OME. If parenteral immunoglobulin treatment is studied, stringent controls, carefully designed protocols, and clinically relevant outcome measures are necessary.

7. To what degree are practitioners still treating prolonged MEE after AOM with antibiotics? Some articles reviewed herein suggest it remains prevalent. Does existing evidence preclude a benefit from prolonged courses of antibiotics? What educational efforts or interventions are necessary to eliminate ineffective attempts to clear MEE with antibiotics?

8. The frequency and significance of subclinical tympanomastoid inflammation in OME and recurrent AOM should be determined, and demographic and clinical predictors of these complications should be identified.

9. Predictors (risk factors) for CSOM should be identified.

10. The safety, tolerability, and efficacy of direct delivery of antimicrobial drugs into the middle ear space in treatment of OM should be investigated in humans, if studies in animal models demonstrate the safety and efficacy of this therapeutic approach.

11. Additional studies are urgently needed to define the change that occurs in nasopharyngeal microflora during long-term (4 weeks or greater) antimicrobial prophylaxis or treatment of OM in order to identify the frequency of antimicrobial resistance during treatment. Such nasopharyngeal surveillance should be a part of all new antimicrobial drug clinical trials.

12. Controlled trials are needed to study the effect of risk factor modification, including behavioral (eg, feeding practices and sleep position) and environmental (eg, passive smoke exposure and day-care attendance) interventions, on OM incidence and severity.

13. Putative relationships among sinus, adenoid, and middle ear inflammations should be studied, especially with regard to indications for treatment of sinusitis or adenoiditis in children with recurrent AOM and OME.

14. Putative associations between allergy and frequent OM need further definition. If associations are found, studies directed at allergy treatment are warranted.

15. Studies of mucolytic agents should be expanded to include investigation of mucus production. Studies in animal models demonstrating safety and efficacy are needed before proceeding with clinical trials.

16. An informational, nonregulatory, worldwide central registry of OM clinical trials is needed to facilitate international communication regarding studies in progress and issues of experimental design.

17. Is treatment of OME with antibiotics ever justified? Given the small, short-term benefits observed in blinded RCTs (eg, a 14% treatment advantage), when is treatment justified? Can we identify subsets of children based on clinical or demographic factors? Do children with special needs who are intolerant of even transient MEE (eg, children with developmental delays, learning problems, baseline sensorineural hearing loss, cognitive impairment) benefit from therapy? Only a large, controlled, prospective study could address these issues. Given the relatively small effect of antimicrobial treatment on the clinical course of chronic OME, all clinical trials of this treatment should be placebo-controlled.

18. Is treatment of OME with oral corticosteroids ever justified? Existing RCTs are small, and meta-analyses suggest a promising effect that just misses statistical significance. Larger, well-designed studies are needed to increase precision and statistical power. These trials must include long-term follow-up, since the treatment benefit appears to be relatively short-term in existing studies.

19. Meta-analysis suggests a potential impact of mucoactive therapy on OME, but existing trials are of small size, use nonuniform outcome measures, and vary greatly in quality. More research is needed in this area to better define the extent and duration of treatment benefit.

20. Meta-analysis suggests a potential impact of autoinflation on OME, but existing trials vary greatly in quality, and the pooled sample size remains small. More research is needed in this area to better define the extent and duration of treatment benefit.

21. Given the high natural cure rate of AOM and most OME, studies of alternative or complementary medical therapies should have appropriate concurrent controls.

22. Future studies of antimicrobial prophylaxis for prevention of AOM should be monitored carefully to identify the benefits of therapy and the potential risk of accelerated bacterial resistance. Do agents other than amoxicillin (eg, sulfisoxazole) pose less risk for bacterial resistance?

23. What is the proper way to statistically combine data from antimicrobial prophylaxis studies of recurrent AOM? Should the primary outcome be episodes per patient-month, or simply episodes per patient?

24. In primary health care, there is a need to evaluate local versus systemic treatment of chronic otorrhea, and similar studies are needed in otolaryngology practice for treatment of chronic otorrhea through tympanostomy tubes.

25. Meta-analysis efforts should be continued to evaluate OM medical and surgical treatments, including cumulative meta-analysis to incorporate results of new trials. Those who use meta-analytic models must be scrupulous to include only studies of appropriate design so as to prevent contamination of results with studies of dubious merit.

26. The indications for and the effectiveness of hearing aids and assistive listening devices should be studied in the treatment of uncomplicated and complicated chronic OM, including measures of functional outcomes.

27. Related to tympanosclerosis and adhesive OM, what type of collagen exists in the tympanic membrane? How do the collagen fibers degenerate dur-

ing formation of adhesive OM, and what factors affect the degenerative process? Is there a genetic predisposition for the acquisition of adhesive OM? What are the causative factors for the formation of tympanosclerosis-myringosclerosis? What chemical mediators are possibly involved in the development of tympanic membrane pathology?

28. How can we predict whether sequelae will occur during treatment of OME, such as tympanostomy tube placement, and how can they be prevented?

SURGICAL

1. Interest in myringotomy for OM has been rekindled by heavy promotion of laser-assisted myringoplasty. What, if any, is the role for this new technique in management of AOM and OME, and how does it relate to the already large body of existing evidence showing inefficacy of myringotomy alone for both conditions? What are the long-term risks and otologic sequelae of laser-assisted myringoplasty, particularly given that proponents of the procedure suggest that multiple applications per ear may be needed?

2. The short-term benefits of myringotomy with intubation (ie, reduced AOM frequency and reduced OME duration during the period of intubation) have been defined with reasonable clarity in a number of recent clinical trials, but its long-term benefits and complications deserve further study, especially in "dry" (intubation for prophylaxis of AOM) ears lacking MEE.

3. What are the quantitative benefits of surgical management for OM? Meta-analysis has not yet been applied successfully in this arena as it has been for medical management.

4. What is the role of adenoidectomy in management of young children (younger than 4 years of age) with recurrent AOM or chronic OME, as either a primary or a secondary procedure? Additional RCTs are needed in this area.

5. What is the impact of tympanostomy tubes or adenoidectomy on health-related quality of life for children with OM? Are objective benefits demonstrated in RCTs perceived and appreciated by parents?

6. Additional studies are needed to compare the efficacy of tympanostomy tubes and antimicrobial drug prophylaxis in the treatment of recurrent AOM. Results of previous trials may not apply in the current environment of multi–drug-resistant OM bacterial pathogens.

7. While "permanent" tympanostomy tubes are not advocated for treatment of uncomplicated chronic OME or recurrent AOM, any future studies of this treatment should carefully define selection criteria and provide long-term follow-up for complications and sequelae of treatment.

8. Can otorrhea in children with tympanostomy tubes be managed with topical antibiotics alone, as suggested by a recent RCT?[31] Which children are best suited for topical therapy alone, without a need for traditional oral antimicrobials?

9. Studies are needed to determine predictors of postintubation otorrhea in cases of chronic OME, and to determine whether this complication results from inflammatory external auditory canal disease, chronic "silent" middle ear disease, or a foreign body reaction to the tube.

10. Studies are needed to evaluate the efficacy of adenoidectomy for chronic OME treatment in children younger than 4 years of age; to develop selection criteria for adenoidectomy to differentiate patients most likely to benefit from the procedure; and to measure the effect of adenoidectomy timing, with respect to OM severity and duration, on long-term outcome.

11. The role of exploratory tympanotomy, mastoidectomy, and middle ear reconstruction in recurrent AOM, chronic OME, and CSOM should be investigated, since subclinical tympanomastoid inflammation may be unresponsive to conventional short-term medical management, particularly in areas of anatomic obstruction within the middle ear and mastoid. The use of radiographic imaging in this regard should be studied.

12. Studies are needed to assess the role of intubation during tympanoplasty procedures to avoid postoperative atelectasis.

13. Studies are needed to improve detection and treatment of inner ear sequelae of OM, such as sensorineural hearing loss and endolymphatic hydrops, and to improve detection and treatment of associated round window membrane disease.

14. Studies are needed to measure the effect of surgical treatment of retraction pockets and atelectasis on middle ear sequelae.

15. Studies of autoinflation would be of value in determining the effect of this treatment on tubal dysfunction before surgery.

16. What surgical procedures for adhesive OM ensure improvement in postoperative hearing outcomes?

HUMAN TEMPORAL BONE RESEARCH

1. Human temporal bone histopathology studies are needed to identify tympanic membrane pathol-

ogy (eg, intra–tympanic membrane cholesteatoma, myringosclerosis, retraction pocket, perforation, fluid-filled cystic space) associated with common surgical procedures (eg, polyethylene tube insertion, paracentesis, and tympanoplasty).

2. Development of diagnostic tools would be of value, such as multifrequency tympanometry and otoacoustic emissions to complement otoscopy for diagnosis of middle ear pathology — especially in a tympanic membrane that appears "normal" (silent OM).

3. Since the frequency of occurrence of OM is elevated in patients with cleft palate, labyrinthitis secondary to OM may be more prevalent in cleft palate patients than in non–cleft palate patients. A human temporal bone histopathology analysis of this correlation may be of significant contribution to the care of the patient with cleft palate.

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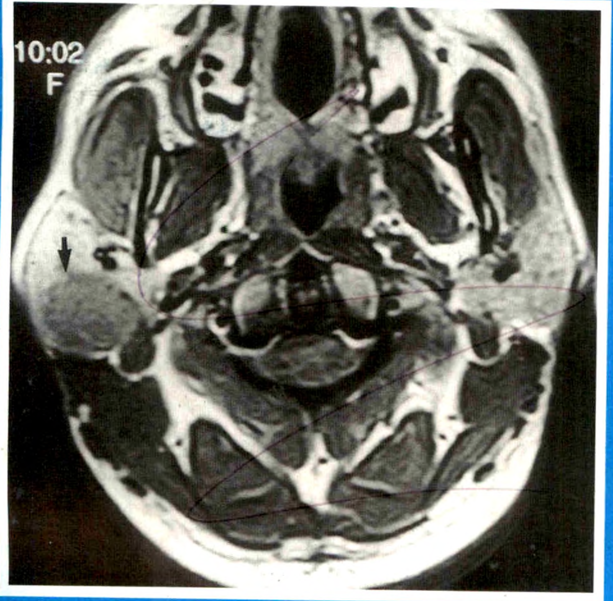

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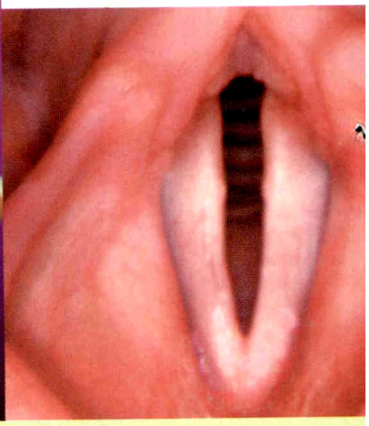

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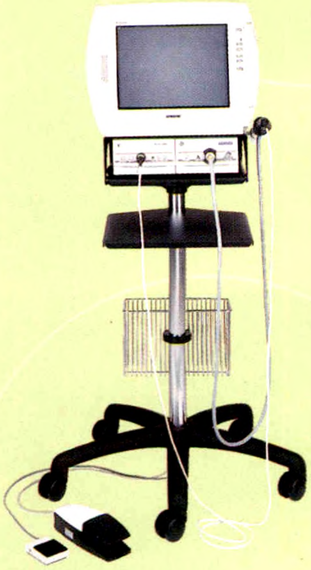

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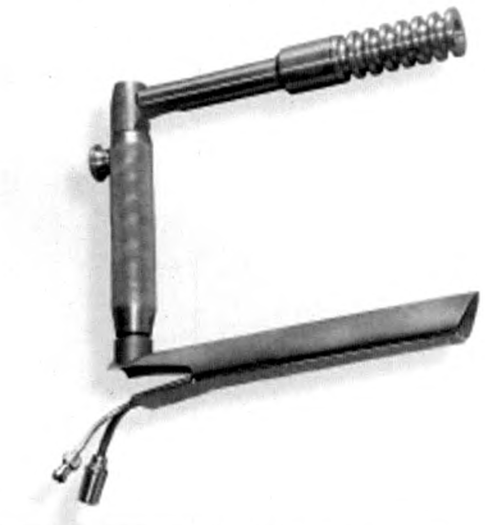

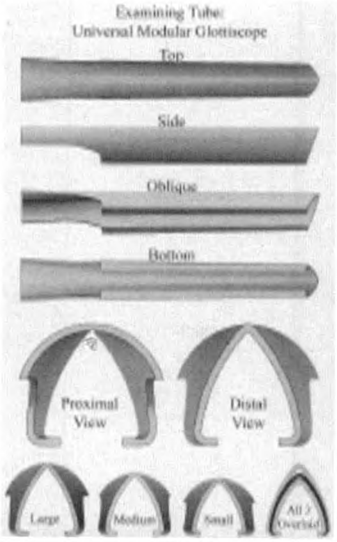

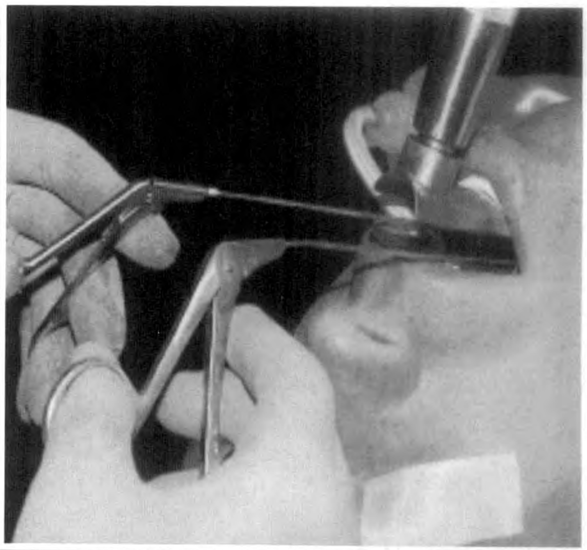

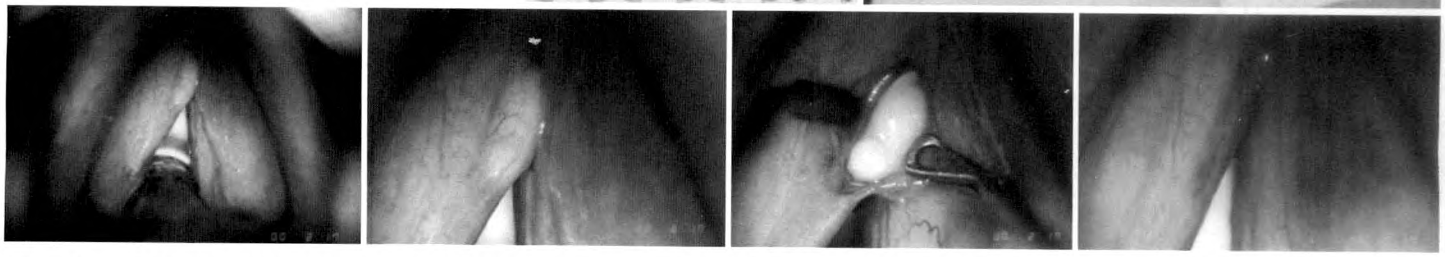

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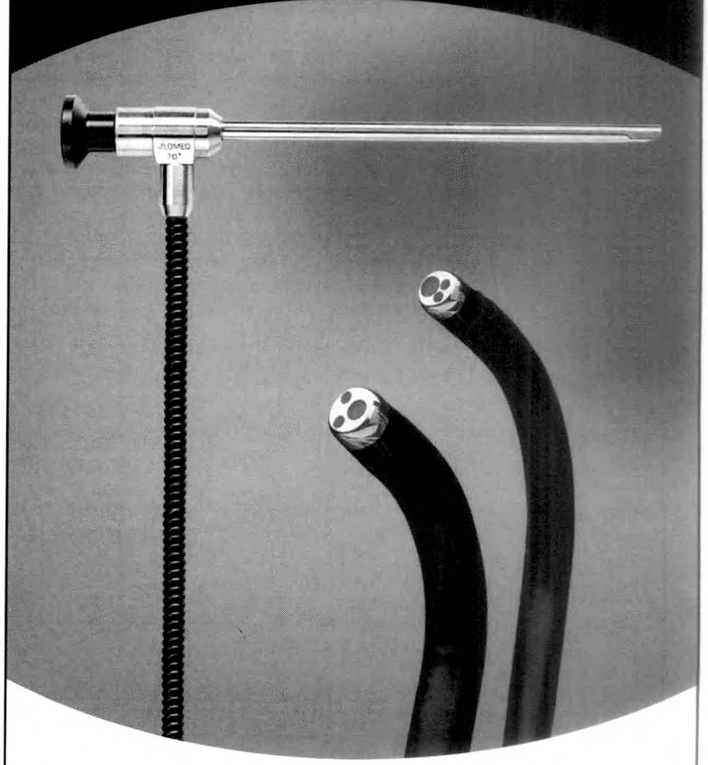

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METALLIC TRACHEAL STENTS: COMPLICATIONS ASSOCIATED WITH LONG-TERM USE IN THE UPPER AIRWAY

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The use of metallic airway stents for the treatment of benign airway stenosis is increasingly advocated; however, the long-term safety and efficacy of these devices has not been established. Three case studies involving late but significant proximal tracheal stent complications are reported: 1 related to proximal mechanical stent failure and 2 related to obstructing granulation tissue and stenosis at the proximal stent orifice. Placement of these stents in close proximity to the cricotracheal junction is thought to be primarily responsible for these complications as a result of the excess shearing forces created at the stent-mucosal interface by the differential motion of the stent relative to the rigid subglottic airway and the more distensible trachea. Although the use of metallic stents remains appropriate in cases in which there is a defined and relatively short-term end point for treatment, caution is urged in using them in the proximal trachea for long-term management of benign airway disease.

KEY WORDS — relapsing polychondritis, tracheal stenosis, tracheal stent, tracheomalacia.

INTRODUCTION

Symptomatic proximal tracheal stenosis can be treated with segmental resection and reanastomosis, various forms of tracheoplasty, often with cartilage grafting, or, as has been more recently reported, intraluminal placement of covered or uncovered metal mesh stents. Given the surgical accessibility of this portion of the airway, this latter option has historically been considered as a temporary method of airway maintenance to be used until a more definitive treatment can be performed, or as a form of permanent palliation in cases such as terminal cancer in which there is limited life expectancy. More recently, however, perhaps on the basis of an encouraging experience in the lower airway,[1-5] and despite a lack of studies that establish their long-term safety and efficacy in this situation, these same stents, designed primarily for lower airway use, have been advocated as a form of permanent treatment for benign proximal tracheal stenosis.

The purpose of this report is to describe 3 late but significant complications that occurred as a result of the use of metallic tracheal stents in the proximal airway for benign tracheal stenosis, and to quantitatively review the reported complications of metallic airway stents.

CASE REPORTS

Case 1. A 52-year-old woman presented with stridor, increased difficulty in swallowing, and pain in her lower neck and upper back. The patient's pertinent medical history included severe pneumonia 25 years previously, which required prolonged ventilatory support, facilitated by tracheotomy, for a period of $2^1/2$ weeks. A stenosis subsequently developed at the tracheotomy site, approximately 3.5 cm below the level of the true vocal cords. Over the ensuing 15 years, the patient had undergone various forms of dilation, laser surgery, and open repair without success. Eight years before the present admission, she underwent placement of a Gianturco Z-stent (Cook Inc, Bloomington, Indiana) without complication (15 × 60-mm stent placed 2 cm below the true vocal cords and extending a distance of 6 cm). Her stridor and dyspnea resolved with the stent placement, although she did have a persistent cough.

A chest radiograph performed at the time of her current admission revealed that 4 wires had dissociated themselves from the main body of the stent (Fig 1). A computed tomographic scan showed marked tracheal stenosis at the proximal margin of the stent over a distance of 2 cm, with a minimal luminal diameter of 5 × 7 mm. Two wires appeared to transgress the lower cervical esophagus, with marked inflammation of the surrounding soft tissues (Fig 2). The distal stent appeared intact, with no evidence of airway compromise (Fig 3). Extravasation of contrast dye was seen on barium swallow study at the

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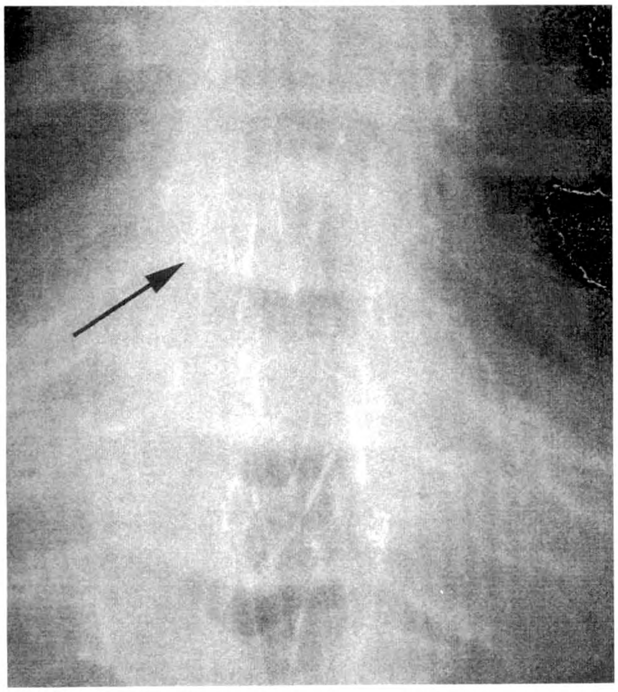

Fig 1. Chest radiograph shows fracture of Gianturco stent and migration into adjacent tissues.

site of one of the wires. Rigid bronchoscopy showed 2 shelves protruding into the airway at the upper edge of the stent, causing a 70% airway stenosis. On rigid esophagoscopy, a wire was noted extruding through the anterior wall of her esophagus 4 cm distal to the esophageal introitus. It was removed endoscopically; the patient's pain resolved, and the radiographic evidence of paraesophageal inflammation cleared. A tracheoplasty was subsequently performed with costal cartilage grafting. The findings at the time of surgery were that the upper portion of the stent was disrupted, with wires protruding through the anterior and posterior tracheal walls; the distal stent, however, was deeply incorporated into the tracheal wall and was mucosalized with a minimum of overlying inflammation. The upper stent was removed. At 1 year, the patient is asymptomatic and has no evidence of restenosis by endoscopy.

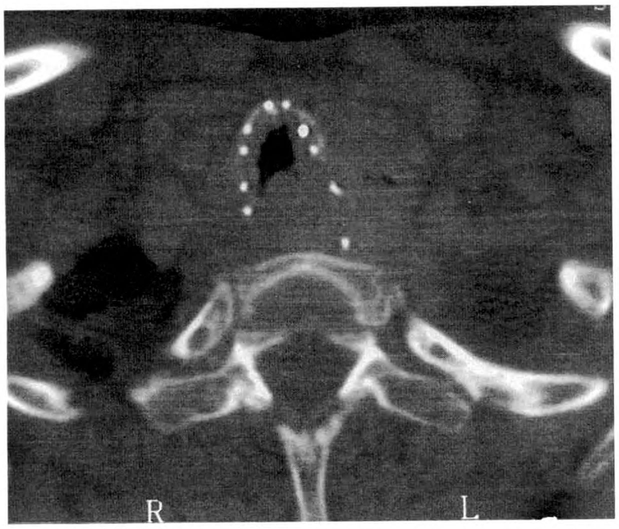

Fig 2. Computed tomographic scan shows migration of proximal stent wires outside tracheal lumen into esophagus. Note that tracheal lumen is narrowed by inflammation and granulation tissue.

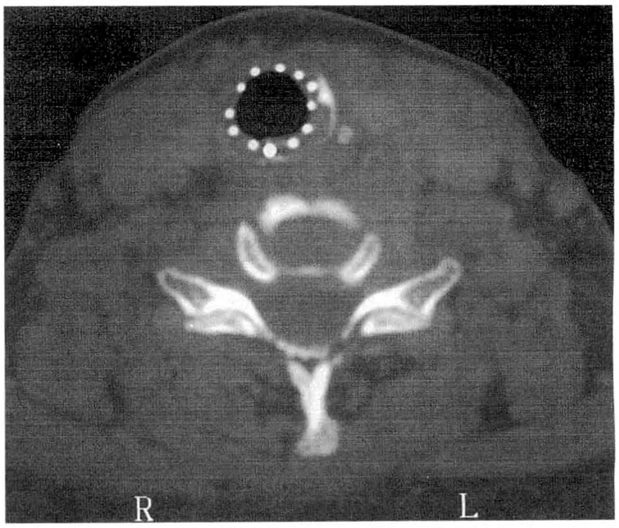

Fig 3. Computed tomographic scan shows incorporation of distal stent into tracheal wall with no evidence of mucosal swelling or airway compromise.

Case 2. A 48-year-old man presented with significant stridor, cough, and difficulty clearing his secretions. The patient's pertinent medical history included a 10-year history of intermittent inflammation of the conchal cartilage of his ears, as well as significant diffuse tracheomalacia and bronchomalacia documented endoscopically, as well as by dynamic computed tomographic scanning. On the basis of the findings and history, relapsing polychondritis was diagnosed. Treatment with steroids and antibiotics had not improved his pulmonary symptoms. Bronchoscopic examination had revealed dynamic lateral collapse of the tracheal lumen of as much as 60% and 90% collapse of the left main stem bronchus with coughing. Metal mesh stents (Wallstent, Boston Scientific Corp, Natick, Massachusetts) were placed in his left main stem bronchus and trachea, the stent in the latter location beginning approximately 2.5 cm below the level of his true vocal cords. The patient's symptoms resolved. As seen by endoscopy 4 months after placement, the tracheal stent was well incorporated into the tracheal wall over all but the most proximal 2.0 cm of its length, where exposed wire and granulation tissue could still be seen (Fig 4).

Approximately 12 months after stenting, at the time of his initial presentation to our institution, the patient had severe stridor. Flexible fiberoptic laryngoscopy revealed a near-complete airway stenosis

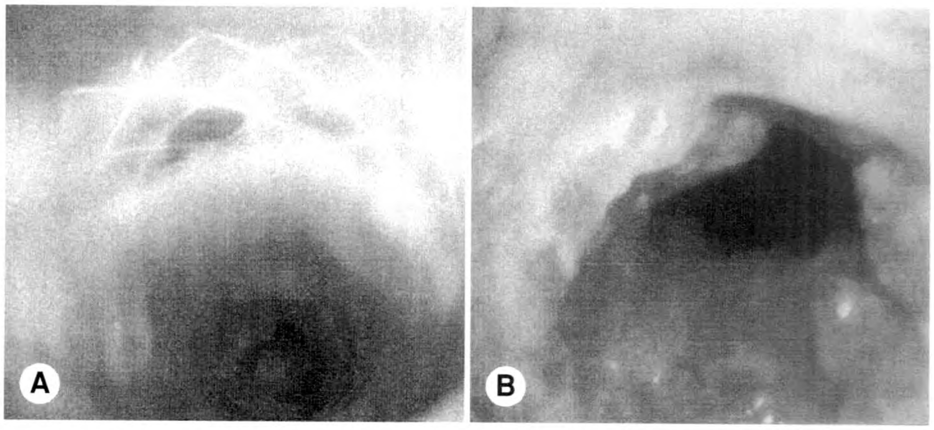

Fig 4. Endoscopic view of proximal stent orifice with both **A**) nonmucosalized metal stent framework and **B**) granulation tissue.

2.5 cm below the glottis, consisting of 2 slightly offset soft tissue shelves at the proximal end of the stent (Fig 5). In contrast, the distal end of the tracheal stent and the left main stem stent showed minimal evidence of inflammation. Initial management consisted of bronchoscopic removal of a portion of these shelves. Definitive management was accomplished with open tracheoplasty. The findings at the time of surgery were that the proximal 2 cm of the stent was loose in the airway (ie, not incorporated into the mucosa) and was surrounded by granulation tissue and inflammation, accounting for the shelves that were seen in the airway. It was amputated horizontally at the point at which it was fixed submucosally, and the trachea closed back on itself after removal of the majority of the granulation tissue. This procedure resulted in complete resolution of the patient's symptoms, and an endoscopic examination performed 1 year after surgery revealed that the airway was not inflamed and was approximately 80% of its normal size.

Case 3. A 47-year-old woman presented with severe biphasic stridor. Her medical history included a severe injury sustained in a motor vehicle accident; long-term ventilation and tracheotomy were required, and she was eventually decannulated 12 years after her injury. Two years after decannulation, an upper tracheal stenosis developed at the site of her tracheotomy cuff; it was corrected by open repair, but the details are not available for review. Sixteen years later, she presented to an outside hospital with severe stridor and was found to have a significant airway stenosis and collapse measuring 4 cm in length at the site of her previous tracheotomy. Two metal mesh stents (Wallstent, 14 × 40 mm and 14 × 60 mm) were placed, beginning approximately 2.5 cm below the level of the true vocal cords and extending down the trachea to within 1 cm of the carina. The patient's symptoms resolved.

Two years later, the patient presented to our institution with a 6-month history of increasing stridor, dyspnea, and a subjective sensation of "something flipping in and out of [her] windpipe." Flexible fiberoptic endoscopy revealed polypoid granulation tissue occluding approximately 70% of her airway and surrounding the upper margin of the stent approximately 2.5 cm below the level of the true vocal cords. The granulation tissue was removed by rigid bronchoscopy. The distal stent appeared to be well incorporated into the tracheal wall without inflammation. Removal of the granulation tissue was performed on 2 occasions, but it recurred within 2 to 3 months each time. Definitive repair was therefore undertaken. The upper 3 cm of the stent, which was "loose" in the airway (nonmucosalized), was amputated and removed. The collapsed, fibrotic, and

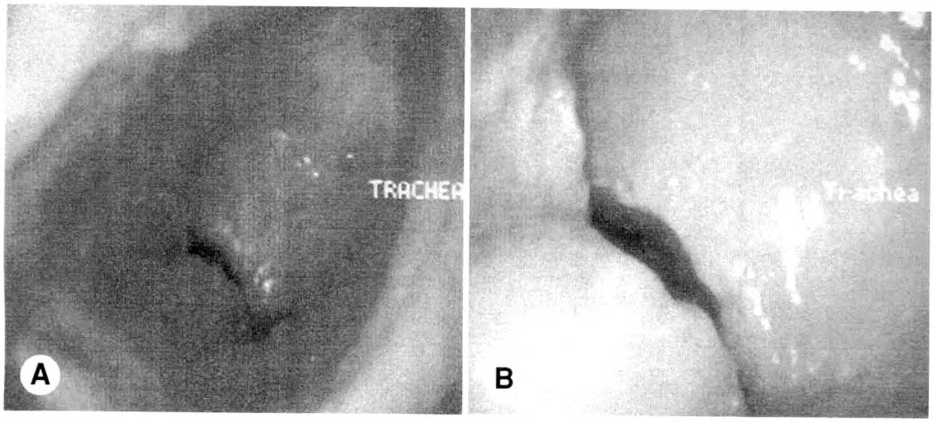

Fig 5. Endoscopic view of subglottic–upper tracheal "shelves" of inflammation and granulation tissue at proximal stent orifice seen **A**) from level of true vocal cords and **B**) in close-up.

TABLE 1. SUMMARY OF REPORTED COMPLICATIONS BY STENT TYPE

Stent Type	*No. of Articles*	*No. of Patients*	*Complications* No.	*Complications* %
Gianturco	20	228	77	34
Palmaz	6	68	37	54
Wallstent	6	27	9	22
Metallic*	13	174	49	28

*Type not specified in article, or multiple types reported in article and complications not attributed to specific type.

TABLE 2. SUMMARY OF COMPLICATIONS

Complication	*No.*	*% of Total Complications*
Granulation tissue or granuloma	47	27
Restenosis	33	19
Migration	17	10
Fracture	13	8
Erosion	6	4
Bleeding	2	1
Infection	14	8
Technical	18	11
Death	13	8
Other	9	5
Total	172	

scarred anterior cricoid cartilage and upper trachea underlying this portion of the stent were resected, and the distal trachea (the upper centimeter of which had had the stent removed) was then anastomosed to the thyroid cartilage anteriorly and cricoid lamina posteriorly as in standard cricotracheal resection techniques. The remaining distal stent that was incorporated into the tracheal wall was not disturbed. The patient was extubated in the operating room, and over the past 12 months, she has maintained a stable, noninflamed airway that is approximately 80% of its normal size.

Literature Review. Relevant articles were found with a Medline search (1966 to October 1999) for the key word "tracheal stenosis" and the text word "stent." The search was limited to English-language journals and human subjects. This search yielded 97 articles, from which 45 were chosen for review on the basis of relevant content. Only articles pertaining to metal mesh stents were reviewed. These included Gianturco, Wallstent, Palmaz, and other metallic stents not otherwise specified in the article. The articles were reviewed for number of patients, position of stent placement, type of stent, maximum follow-up, and complications. Complications were classified as granulation tissue or granulomas, restenosis, stent migration, stent fracture, erosion outside the tracheal or bronchial lumen, infection, bleeding, technical complications, and death. In cases of individual patients with more than 1 complication, the complications were counted separately. Cases of death were included if they occurred within 24 hours of stent placement or resulted from a complication attributable to the stent. Technical complications included improper placement requiring repositioning or replacement, mucosal laceration, failure of the stent to relieve the obstruction, and stent disruption from external causes such as tracheal suctioning.

Every effort was made to determine the stent position relative to the true vocal cords. Unfortunately, this was rarely mentioned, perhaps because the vast majority of articles come from the pulmonary, thoracic surgery, or radiology literature, in which landmarks relative to the tracheobronchial tree anatomy are used instead. However, even in those articles published in the otolaryngology literature,[1,2] this information is often lacking.

RESULTS

There were 172 complications reported in 497 patients (34%). The median follow-up was 16 months, with a maximum of 53 months. The results are reported in Tables 1 and 2. There was no significant difference in the overall incidence of or types of complications with any particular type of stent.

DISCUSSION

Airway stenting is accepted as a viable option in situations in which there is a defined and relatively short-term end point for treatment, in patients with larger airway obstruction as a result of malignancy, and as a temporizing measure for patients awaiting definitive surgical intervention.[1,3,5-15] However, the longest period of follow-up in the literature review reported here was 52 weeks.[4] Despite or perhaps because of this lack of long-term safety data, stenting is being increasingly advocated for the long-term treatment of benign tracheal stenosis, both primarily and after failure of other therapeutic methods.[1-5,16] The cases presented here provide evidence that complications may develop in the proximal airway even after years of successful stenting, suggesting the need for a more cautious approach.

Although the nature of the complication in case 1 (stent disruption) was different from those in cases 2 and 3 (stenosis and granulation tissue at the proximal stent orifice), all complications occurred in the most proximal part of the trachea, at its junction with the cricoid cartilage. Metallic mesh stents are distensible to a degree, in keeping with the distensibility of the trachea, for which they were designed. In contrast, the subglottic airway, with its intact cricoid ring, is not. The junction of these rigid and semirigid airways (cricotracheal junction) is therefore subject to a larger degree of motion than the rest of the tracheobronchial tree, in the form of the flexion-extension and distention-contraction that occur with head motion and respiration, respectively. It is hypothesized that placement of these stents in close proximity to the site of excess motion at this cricotracheal junction is primarily responsible for the long-term complications reported here. The vulnerability of this location is thought to be due to the excess shearing forces created at the stent-mucosal interface by the differential motion of the stent relative to the rigid subglottic airway and the more distensible trachea. These shearing forces prevent stent incorporation and mucosalization, may lead to ongoing mucosal injury and granulation tissue, and ultimately can cause stent fracture or restenosis around the stent orifice. Unfortunately, the literature does not contain sufficient information about the relative position of the stent to the cricoid cartilage for us to determine the degree to which this occurs.

Fracture of Gianturco stents has been previously reported with variable consequences. Hramiec and Haasler[17] reported 2 cases of stent fracture that occurred 2 and 6 months after tracheal stenting. In the latter case, the stent wire extruded from the trachea adjacent to the right subclavian artery. Other cases of spontaneous stent fracture without serious sequelae have also been reported.[18,19] However, there are also reports of fracture with brachiocephalic artery erosion that resulted in massive hemoptysis[19,20] and death.[21] The proximal location of the fracture in the case reported here prevented these more serious problems.

Operation, and especially reoperation, for proximal tracheal and subglottic stenosis can be technically challenging, but has a high chance of long-term success in experienced hands,[22] particularly with the increased use of cricotracheal resection. Therefore, despite the appeal of the relative simplicity of stenting techniques, the potential for complications such as those seen here would suggest that this technique should not be used as a long-term solution for problems in the proximal airway, in which the stent will need to be placed across or adjacent to (within 1 cm) the cricotracheal junction.

CONCLUSIONS

Although the use of metallic stents in the airway remains appropriate in cases in which there is a defined and relatively short-term end point for treatment, caution is urged in using them for long-term management of benign airway disease in the proximal trachea, in which the stent will need to be placed across or adjacent to (within 1 cm) the cricotracheal junction.

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SWALLOWING AND SENSATION: EVALUATION OF DEGLUTITION IN THE ANESTHETIZED LARYNX

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To better characterize the role of laryngeal sensory systems in swallowing, we assessed 5 healthy adult volunteers via functional endoscopic evaluation of swallowing before and after bilateral superior laryngeal nerve block. The volunteers were tested multiple times with both liquid and puree consistencies. Laryngeal anesthesia resulted in a significantly higher ($p < .05$) incidence of premature spillage, pharyngeal residual, and laryngeal penetration with all consistencies, and a higher incidence of tracheal aspiration with liquid. Aspiration of puree was increased, but the difference did not achieve significance. These results contrast with those achieved by others with topical anesthesia, which caused little impairment. Other afferent modalities besides light touch, interrupted by superior laryngeal nerve block but not by topical anesthesia, may account for the difference. It is likely that this study underestimates the severity of clinical disability from afferent deficits, because of the short duration of the experimental deficit and the lack of associated comorbidities in the subjects. These findings have implications for clinical sensory testing and sensate flap reconstruction of pharyngeal defects.

KEY WORDS — laryngeal sensation, sensory testing, superior laryngeal nerve, swallowing.

INTRODUCTION

Sensation has long been assumed to play a critical part in swallowing. Until recently, only crude means have been available for testing the sensory function of the larynx and the pharynx in humans. Often, these amounted to the touch of an instrument to the mucosal surface in question. The results were unquantifiable, and only the grossest defects were detectable.

Since 1993, a clinically practical means of testing laryngeal sensation has been available. It involves the delivery of air pulses of measured pressure to the mucosa of the larynx.[1] By observing either the patient response or, in the case of a patient with altered consciousness, activation of the laryngeal adductor reflex, sensation can be quantified, and differences between the left and right sides can be identified. Normative data have been established, and laryngopharyngeal sensation has been shown to decrease with age.[2,3] Furthermore, measured sensory deficits have been correlated to observed swallowing dysfunction and the development of aspiration pneumonia in selected patient populations.[4,5]

Many patients studied systematically by this means have had neurologic injury, often with associated cognitive, motor, and coordination deficits. The role of sensation alone in the dysphagia of this population is unclear and has recently come under question.[6] This study was undertaken to better characterize this. Understanding the role of sensation in swallowing is important, not only for our general understanding of the physiology of deglutition, but also for practical clinical reasons. It has major implications for ongoing efforts to reconstruct head and neck defects with sensate flaps, and for the value of sensory testing in the evaluation of dysphagia.

PATIENTS AND METHODS

After informed consent was obtained, 5 healthy adult volunteers, 26 to 35 years of age, underwent fiberoptic endoscopic examination of swallowing (FEES) in both normal and anesthetized states, thus serving as their own controls. At initial examination, basic upper aerodigestive tract anatomy and function was evaluated, including velopharyngeal closure and vocal fold motion. With no anesthesia, each subject performed 3 individual swallows of puree consistency and 3 of thin liquid consistency. Each swallow was examined for 4 abnormal findings: premature spillage, pharyngeal residual, laryngeal penetration, and tracheal aspiration. These were each rated simply as present or absent. Care was taken not to cue the subject to the findings during the evaluation.

The subject then underwent anesthesia. We deliberately duplicated our procedure for office surgery. This has been fully described elsewhere.[7] In brief, it consists of bilateral superior laryngeal nerve block

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TABLE 1. RESULTS OF FUNCTIONAL ENDOSCOPIC EVALUATION OF SWALLOWING BEFORE AND AFTER ANESTHESIA

	Premature Spillage (n = 5)		*Pharyngeal Residual (n = 5)*		*Laryngeal Penetration (n = 5)*		*Tracheal Aspiration (n = 5)*	
Type of Swallow	*Control*	*Anesthesia*	*Control*	*Anesthesia*	*Control*	*Anesthesia*	*Control*	*Anesthesia*
Puree	2	3	2	5	0	2	0	1
Puree	0	3	3	4	0	0	0	1
Puree	0	3	1	5	0	4	0	1
Thin liquid	0	3	0	4	0	2	0	1
Thin liquid	0	3	1	4	0	4	0	2
Thin liquid	0	3	1	4	0	3	0	1

using 1 mL of 1% lidocaine hydrochloride with 1:100,000 epinephrine on each side, with an additional 1 mL at the thyroid notch, 1 mL of 2% lidocaine hydrochloride injected intratracheally via the cricothyroid membrane, and 4% lidocaine hydrochloride spray delivered perorally to the larynx and pharynx. Before the beginning of swallowing trials, anesthesia was confirmed by passing the fiberoptic laryngoscope through the glottis and touching the subglottic area. The anesthesia was deemed adequate if the subject remained unaware of this maneuver. Anatomy and motor function was again inspected to confirm that no change had occurred as a result of the anesthetic. The subject performed 3 puree and 3 thin-liquid swallows, rated in the same manner as the sensate swallows.

The entire procedure required approximately 30 minutes to complete.

In this manner, 60 swallows were evaluated: 30 in normal subjects and 30 after anesthesia. Fifteen liquid and 15 puree swallows were carried out in each condition.

RESULTS

All subjects demonstrated normal anatomy and function of the upper aerodigestive tract before beginning the swallowing trials. No sensate swallow demonstrated penetration or aspiration, either with puree or liquids. Two sensate swallows, both puree, demonstrated premature spillage of oral contents into the pharynx, and 8 demonstrated pharyngeal residual (6 with puree and 2 with thin liquids; Table 1).

TABLE 2. SUMMARY OF RESULTS

	Sensate Swallows (n = 15)	*Insensate Swallows (n = 15)*	*p**
Puree swallows			
Premature spillage	2	9	<.01
Pharyngeal residual	6	14	<.005
Laryngeal penetration	0	6	<.005
Tracheal aspiration	0	3	<.10
Thin liquid swallows			
Premature spillage	0	9	<.001
Pharyngeal residual	2	12	<.001
Laryngeal penetration	0	9	<.001
Tracheal aspiration	0	4	<.05

*On χ^2 test.

After anesthesia, there was no alteration in anatomy or gross vocal fold motion in any subject. Endoscopic observation of the swallow revealed many abnormal findings (Table 1). Pharyngeal residual was most common, followed by premature spillage. The incidence of each was approximately equal with liquid and puree consistencies. Less common was laryngeal penetration, which occurred more often with liquids, and rarest was tracheal aspiration.

Comparison of the data for sensate and insensate swallows shows anesthetized subjects to have had significantly ($p < .05$) higher incidences of premature spillage, pharyngeal residual, and laryngeal penetration with both consistencies, and of tracheal aspiration with thin liquid. The incidence of aspiration was increased with puree, but the increase did not achieve significance (Table 2).

DISCUSSION

Swallowing is a physiologic event that relies on the detection and integration of a variety of stimuli to produce a complex, coordinated motor response. Intuitively, deficits in the sensory system should produce compromise of the process. Yet, taken as a whole, previous laryngeal anesthesia studies in humans suggest that absent sensation, while undoubtedly altering various parameters of the normal swallow, may not cause clinically significant changes.

Compared to studies in animal models, relatively little experimental observation has been carried out in humans. Using manometry, Månsson and Sandberg[8] observed that topical anesthesia of large areas of the oral and pharyngeal mucosa causes shortening of upper esophageal sphincter opening time and discoordination between upper esophageal sphincter activity and pharyngeal activity. They did not seek to document aspiration and speculated cautiously on the pathological significance of their findings. Ali et al[9] used both videoradiography and manometry to evaluate the swallows of healthy volunteers before and after topical anesthesia. Only pharyngeal contraction times were altered by anesthesia; coordination of swallow events and regional transit and clearance times remained unchanged. Even more striking, no subject was observed to aspirate during deglutition. Most recently, Bastian and Riggs[6] compared the swallows of 13 subjects before and after the administration of topical lidocaine and found little or no difference. Abnormalities, when present, disappeared after the first or second insensate swallow. This was attributed to "cognitive access" to swallowing: swallowing is a stereotyped activity, the steps of which are preserved despite numbness. The authors concluded that "mucosal sensation, considered alone, is a relatively unimportant part of swallowing function."[6(p1975)]

In light of these findings, our attention was drawn to the swallowing function of patients undergoing office surgery under regional laryngeal anesthesia at our center. Penetration and near-aspiration of secretions are consistently observed during such procedures, and the patient often has to be cued to spit or swallow because the operative site, at the level of the glottis, is bathed in saliva. The current study duplicates our anesthetic technique for office surgery in an attempt to recreate the conditions under which gross abnormalities have been seen. The resulting observations, which have given us the impression that sensation is indeed a critical part of deglutition, are different from those reported previously with topical anesthesia.

Two factors may account for these differences. The mammalian larynx is known to contain receptors to a wide variety of stimuli. There are populations of chemoreceptors, mechanoreceptors, and airflow receptors. Within each of these broad categories, there are subclasses. Mechanoreceptors, for instance, may respond to pressure, light touch, or vibration. Deep receptors, not affected by topical anesthesia, may function as proprioceptors for vocal fold position or stretch. It is not clear which of these are present in humans, because it is difficult or impossible to perform the confirmatory electrophysiological tests. Nevertheless, superior laryngeal nerve block, by interrupting the afferent pathway outside the larynx, probably affects a much broader range of receptors than does topical anesthesia. Such a blockade evidently disorders swallowing to a greater extent than does absent mucosal sensation alone. Of note, previous investigators have also proposed such an effect.[9]

In the course of anesthetizing patients for office laryngeal surgery, we have noticed that the occasional patient loses control of pitch when speaking, probably from inadvertent anesthesia of the external branch of the superior laryngeal nerve. Although the cricothyroid muscle is not a principal adductor of the vocal folds, it can aid in closure of the glottis, and its transient denervation may contribute to the more pronounced abnormalities seen with laryngeal nerve block as compared to topical anesthesia. We emphasize, however, that we observed no gross movement abnormalities in patients undergoing the superior laryngeal nerve block technique for a surgical procedure or in the subjects of this investigation. In future studies, electromyography can be used to resolve this issue.

For the same reasons that account for the observed differences between the subjects in our study and those anesthetized topically, we think that superior laryngeal nerve block more closely approximates a clinical sensory deficit. Nevertheless, important differences exist. This study is an introductory investigation of experimentally induced transient sensory deficits in healthy volunteers. Thus, we think it likely that it underestimates the degree of aspiration associated with such deficits in the clinical setting, in which such a condition often results from central nervous system injury associated with serious cognitive or motor problems. Furthermore, we agree with the notion that swallowing is to an extent a stereotyped function, and we hypothesize that a recent lesion is more likely to leave intact the "memory" of a normal swallow than would a long-standing one, and result in correspondingly fewer abnormalities. Under these circumstances, aspiration under superior laryngeal nerve block, even of small quantities, is a significant finding.

CONCLUSION

Laryngeal sensation, which probably consists of more than simply mucosal sensation, is important to swallowing. In 5 healthy volunteers, sensory block of the larynx resulted in significant deterioration of swallowing function, with aspiration observed in some cases. Although care must be taken in extrapolating from our experimental results to clinical situations, the results do support a long-standing belief among clinicians, and suggest that there is a use for clinical sensory testing in identifying deficits and predicting risk. Further, they suggest that efforts to preserve sensation in flaps used in head and neck reconstruction are of potential benefit, and that ongoing investigations into testing of other sensory modalities, and even into sensory reinnervation of the larynx,[10,11] are clinically relevant.

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ADENOVIRUS-MEDIATED EX VIVO GENE TRANSFER OF HUMAN VASCULAR ENDOTHELIAL GROWTH FACTOR IN A RABBIT LARYNGOTRACHEAL RECONSTRUCTION MODEL

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Free autologous cartilage, which is used in laryngotracheal reconstruction (LTR), may undergo progressive necrosis as a result of delayed revascularization. Angiogenic growth factors, such as vascular endothelial growth factor (VEGF), promote angiogenesis in the ischemic environment. We studied the effect of ex vivo gene transfer of VEGF121 on cartilage angiogenesis and graft survival in a rabbit model of LTR. Sixty rabbits underwent LTR with auricular cartilage. The grafts were treated at 1 × 10^9 plaque-forming units with 1) VEGF121 (n = 20), 2) LacZ reporter gene (n = 20), or 3) saline solution (n = 20). Graft neovascularization and survival were histologically assayed at 1 and 10 weeks. Angiogenesis was enhanced at both 1 and 10 weeks after treatment with VEGF121 as compared to controls ($p < .001$). No statistical improvement in graft survival was evident after treatment with VEGF121. Ex vivo gene transfer to cartilage may be a promising gene therapy strategy to enhance revascularization — and, potentially, cartilage survival — under the proper conditions.

KEY WORDS — angiogenesis, gene transfer, laryngotracheal reconstruction, subglottic stenosis, vascular endothelial growth factor.

INTRODUCTION

Reconstruction of the pediatric airway remains a surgical challenge. In the presence of severe laryngeal stenosis, there is a need to provide long-term correction of the compromised lumen to maintain adequate diameter and structural integrity. The ideal graft should be able to survive and provide long-term structural support in the airway. Free autologous cartilage, harvested from the costal margin or auricle, is commonly used as a graft material in pediatric laryngeal expansion surgery. Grafts may be placed in either an anterior position or a posterior position in the larynx. Unfortunately, free cartilage grafts often undergo necrosis and resorption because of inadequate vascularization, poor tissue incorporation, and loss of metabolic support. The subsequent chondrocyte death may result in inadequate structural integrity and airway compromise.

A number of growth factors have demonstrated improvements in wound healing by promoting angiogenesis. Vascular endothelial growth factor (VEGF) may be among the most important of these.[1-5] It is a dimeric glycoprotein with 4 known homodimers (121, 165, 184, and 209 kd). It is directly mitogenic to endothelial cells and induces interstitial collagenase expression.[6,7] In the presence of hypoxia, VEGF messenger RNA (mRNA) is stabilized, transcriptional activation is increased, and angiogenesis appears to be initiated.[8-10] Consequently, VEGF holds potential for improving survival of free grafts in compromised sites by inducing angiogenesis. This hypothesis was examined by measuring the impact of adenovirus-mediated ex vivo gene transfer of VEGF121 on angiogenesis and graft survival in a rabbit laryngotracheal reconstruction model with autologous auricular grafts.

MATERIALS AND METHODS

Adenovirus Preparation. The vectors used in this study were recombinant human serotype 5 adenovirus. (Seed stocks of AdCMVLacZ [adenovirus under control of cytomegalovirus promoter with *Escherichia coli* β-galactosidase transgene] and AdCMV-VEGF121 were obtained from the Vector Core, Institute for Human Gene Therapy, University of Pennsyl-

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From the Division of Pediatric Otolaryngology (Samadi, Jacobs) and the Division of General, Thoracic and Fetal Surgery (Walsh, Bouchard, Crombleholme), The Children's Hospital of Philadelphia, the Department of Otorhinolaryngology–Head and Neck Surgery (Samadi, Jacobs), University of Pennsylvania School of Medicine, and The Wistar Institute (Herlyn), Philadelphia, Pennsylvania. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania Medical Center and The Children's Hospital of Philadelphia.

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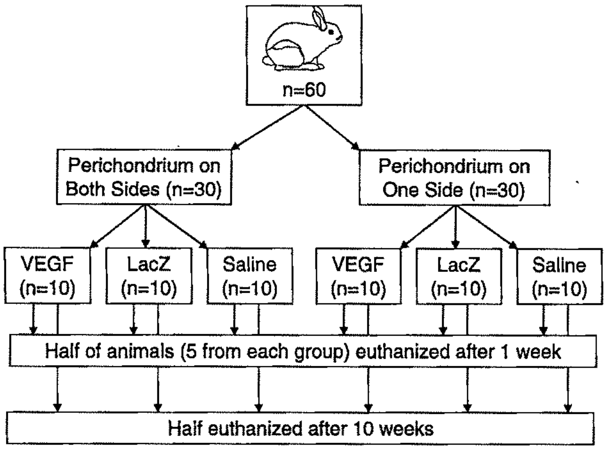
n=60
Perichondrium on Both Sides (n=30)
Perichondrium on One Side (n=30)
VEGF (n=10)
LacZ (n=10)
Saline (n=10)
VEGF (n=10)
LacZ (n=10)
Saline (n=10)
Half of animals (5 from each group) euthanized after 1 week
Half euthanized after 10 weeks

Fig 1. Classification and conditions of experimental animals. VEGF — vascular endothelial growth factor; LacZ — LacZ reporter gene (*Escherichia coli* β-galactosidase transgene).

vania.) The vector was a double-stranded DNA virus that was rendered replication-deficient through deletion of the E1 region of its viral genome as previously described.[11] In AdCMV-VEGF121, the viral E1a and E1b coding sequence has been replaced with an expression cassette consisting of the human VEGF-121 coding sequence driven by a cytomegalovirus promoter. The replication-deficient adenovirus was propagated in human embryonal kidney cells, which complement the viral E1 gene products. After 36 to 48 hours of infection, the virus was recovered in 10 mmol/L Tris buffer through 3 cycles of freezing and thawing. The virus was purified by cesium chloride density centrifugation, dialyzed, and stored at −70°C in 10 mmol/L sodium chloride, pH 8.1, in 10% glycerol. Viral titers were determined by plaque-forming assay.[12] A viral titer of 1×10^9 plaque-forming units (pfu) was chosen on the basis of previous observations, with a ratio of 25 particles to 1 pfu.

Experimental Protocol. All animal procedures were approved by the Institutional Animal Care and Use Committee at The Children's Hospital of Philadelphia in accordance with National Institutes of Health guidelines. Female New Zealand White rabbits (Ace Animals, Boyletown, Pennsylvania) were housed in single cages and fed standard chow and water ad libitum.

The rabbits (4.0 to 4.5 kg) were sedated with intramuscular xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (35 mg/kg) and anesthetized with inhaled 1.5% isofluorane. Using sterile technique, we harvested a 15 × 10-mm auricular cartilage graft with intact perichondrium on one side of the graft (n = 30), or with the perichondrium intact on both sides (n = 30), from the postauricular area of each animal. In 20 animals, the harvested grafts were submerged in an adenoviral construct of VEGF121 at a concentration of 1×10^9 pfu in 400 μL of Dulbecco's modified Eagle's medium solution. Incubations were performed at 5% carbon dioxide and at 37°C. The vector employed has previously been used for successful infection of somatic cells.[13] In the second experimental group (20 animals), the grafts were treated in a similar fashion with the AdCMVLacZ marker virus. A third group of 20 animals served as controls, with grafts submerged in 400 μL of normal saline solution (Fig 1).

After 1 hour of graft incubation, a cervical incision was made and the thyroid and cricoid cartilages of the animal were isolated. An anterior midline laryngofissure was made through the cricoid ring and the upper 2 tracheal rings. A template of the anterior laryngofissure opening was then made for each animal, and the cartilage was carved to conform to the dimensions. The graft was secured to the medial edges of the cricoid and upper tracheal rings by means of 10 to 12 simple interrupted 6-0 Prolene sutures. At the end of the procedure, the rabbits were given positive pressure ventilation to check for air leaks. The wound was irrigated with saline solution. The strap muscles were reapproximated, and the skin was closed with 4-0 Vicryl sutures.

Tissue Processing and Preparation. At 1 week (n = 30) or 10 weeks (n = 30) after the operation, the rabbits were euthanized by barbiturate overdose. The larynx, trachea, and implanted graft were harvested and divided transversely at the center of each graft. The upper half of each specimen was fixed in 10% formalin solution for permanent fixation, paraffin embedding, and sectioning. The lower half was snap-frozen and embedded in Tissue-Tek OCT compound (Sakura Finetek USA, Inc, Torrance, California) for frozen sectioning and immunohistochemical analysis. Five-micron-thick cross sections were stained with hematoxylin-eosin. Immunohistochemical analysis was performed with primary antibodies to platelet/endothelial adhesion molecule (PECAM-1, CD31 monoclonal mouse/anti-human endothelial cell, PharMingen International, San Diego, California) and trichrome stain. Whole mount photomicrographs of the hematoxylin-eosin, PECAM-1, and trichrome stains were obtained.

PECAM-1 Histologic Assessment. We used PECAM-1 immunohistochemical staining to assess the degree of angiogenesis in our grafts. The PECAM-1–stained slides underwent quantitative immunohistologic and morphometric analysis with a Leica DMR microscope camera system (Wetzlar, Germany) and the Image-Pro Plus analysis software from Media Cybernetics (Silver Spring, Maryland). A complete montage of the extraluminal surface of the PECAM-1 slide was reconstructed (one 10× power field around the perimeter of the cartilage graft). Angiogenesis was quantified by manually counting the number of blood vessels in histologic cross section (per square millimeter) in tissues within one 10× power field above the extraluminal surface of each graft.

Trichrome Histologic Assessment. Trichrome staining was used to assess cartilage survival, necrosis, and neochondrification. Trichrome-stained slides similarly underwent morphometric analysis with the Leica DMR microscope camera system and the Image-Pro Plus analysis software from Media Cybernetics. A complete montage of the trichrome slide was reconstructed that encompassed the full length of the graft. Viable cartilage was characterized by chondrocytes containing nuclear material with full preservation of inherent structural integrity. Cartilage necrosis was defined as loss of nuclear material within the chondrocytes. Neochondrification was illustrated by chondrocytes that had a modified structural integrity while maintaining their nuclear material. Areas of viable cartilage, necrosis, and neochondrification were each separately measured and analyzed.

Statistical Analysis. Statistical significance was determined by 3-way (perichondrium × virus × survival time) analyses of variance. Because of the many possible comparisons, a criterion of $p < .05$ was used for significance.

RESULTS

All 60 rabbits survived the entire postoperative period, and there were no major complications. One rabbit developed mild stridor on the first postoperative day that spontaneously resolved over a 3-hour period. Upper airway obstruction did not occur in the other animals. One rabbit developed a small, localized area of superficial wound dehiscence that healed spontaneously. There was no loss of structural integrity of the tracheal reconstruction at the time of sacrifice. The reconstructed montages of all 60 grafts were satisfactory for immunohistochemical and morphometric analysis.

Angiogenesis. Histologically, angiogenesis was primarily identified on the extraluminal surface of the graft, with minimal angiogenesis seen on the luminal side (Fig 2). Morphometric analysis, at 1 week after operation, revealed that the double perichondrial grafts treated with AdCMV-VEGF121 ($n = 5$) developed 96 ± 36 (mean ± SD) vessels per square millimeter, as compared to 28 ± 8 vessels per square millimeter for those treated with AdCMVLacZ ($n = 5$) and 18 ± 14 vessels per square millimeter for the group treated with saline solution ($n = 5$; $p < .001$). A similar pattern was observed in the single perichondrial grafts 1 week after operation, with the grafts treated with AdCMV-VEGF121 ($n = 5$) demonstrating the most enhanced pattern of angiogenesis at 57 ± 2 vessels per square millimeter, compared to 21 ± 5 vessels per square millimeter and 19 ± 14 vessels per square millimeter for the grafts treated with AdCMVLacZ ($n = 5$) and saline solution ($n = 5$), respectively (Fig 3).

Comparable results were observed at 10 weeks after operation: the double perichondrial grafts treated with AdCMV-VEGF121 ($n = 5$) demonstrated increased neovascularization at 80 ± 25 vessels per square millimeter, as compared to 33 ± 9 vessels per square millimeter for those treated with AdCMVLacZ ($n = 5$) and 11 ± 5 vessels per square millimeter for the group treated with saline solution ($n = 5$; $p < .001$; Fig 2). At 10 weeks, the single perichondrial grafts treated with AdCMV-VEGF121 ($n = 5$) similarly demonstrated 44 ± 20 vessels per square millimeter, as compared to 25 ± 12 vessels per square millimeter in the grafts treated with AdCMVLacZ ($n = 5$) and 21 ± 7 vessels per square millimeter in the saline-treated control group ($n = 5$; Fig 3). The analysis of variance confirmed a significant effect of AdCMV-VEGF121 treatment ($p < .001$), confirming the efficacy of VEGF121. Angiogenesis was also significantly higher in double perichondrial grafts as compared to single perichondrial grafts ($p < .003$). There was no significant change in angiogenesis from week 1 to week 10 ($p = .389$). Similarly, no statistical difference in angiogenesis was observed between the AdCMVLacZ-treated grafts and the saline-treated grafts ($p = .210$).

Graft Viability. Graft survival, as measured by trichrome staining, demonstrated greater viability in all 1-week cartilage grafts ($n = 30$) than in the 10-week cartilage grafts ($n = 30$; $p < .0001$; Fig 4). At 1 week after operation, the double perichondrial grafts treated with AdCMV-VEGF121 ($n = 5$) demonstrated 2.27 ± 0.58 mm^2 of viable cartilage, as compared to 2.67 ± 0.34 mm^2 for those treated with AdCMVLacZ ($n = 5$) and 1.92 ± 0.33 mm^2 for the group treated with saline solution ($n = 5$; $p = .481$). Similarly, the single perichondrial grafts treated with AdCMV-VEGF121 ($n = 5$) showed, at 1 week after operation, 2.34 ± 0.16 mm^2 of viability, as compared to 2.11 ± 0.20 mm^2 and 2.04 ± 0.46 mm^2 for grafts treated with AdCMVLacZ ($n = 5$) and saline solution ($n = 5$), respectively ($p = .593$; Fig 5).

At 10 weeks after operation, the double perichondrial grafts treated with AdCMV-VEGF121 ($n = 5$) demonstrated 1.49 ± 0.99 mm^2 of viable cartilage,

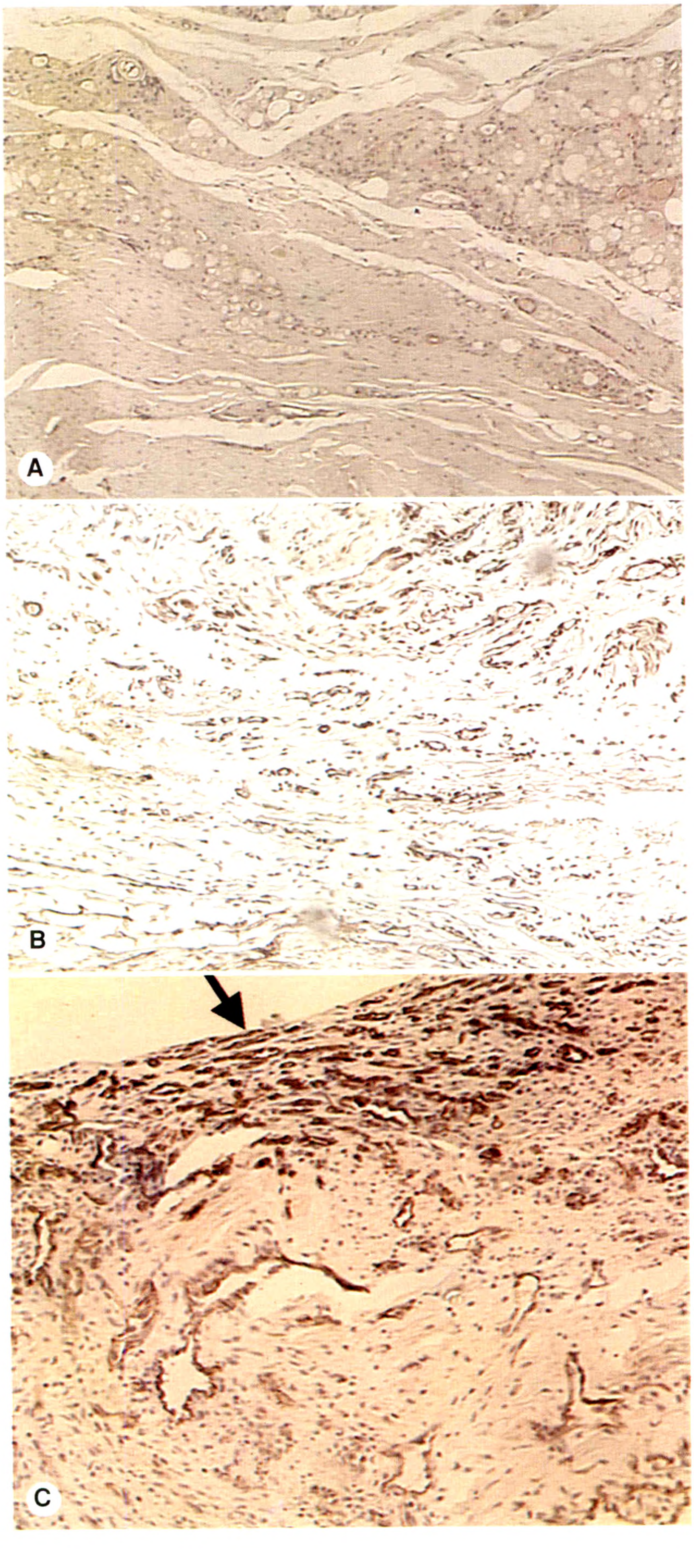

Fig 2. Photomicrographs of PECAM-1 (platelet/endothelial adhesion molecule) stains in double perichondrial grafts at 10 weeks after operation. **A)** Graft treated with saline solution. **B)** Graft treated with AdCMVLacZ (adenovirus under control of cytomegalovirus promoter with *E coli* β-galactosidase transgene). **C)** Graft treated with AdCMV-VEGF$_{121}$ (adenovirus under control of cytomegalovirus promoter with *E coli* VEGF$_{121}$ transgene). Note intense staining of vessels on extraluminal surface of graft (arrow).

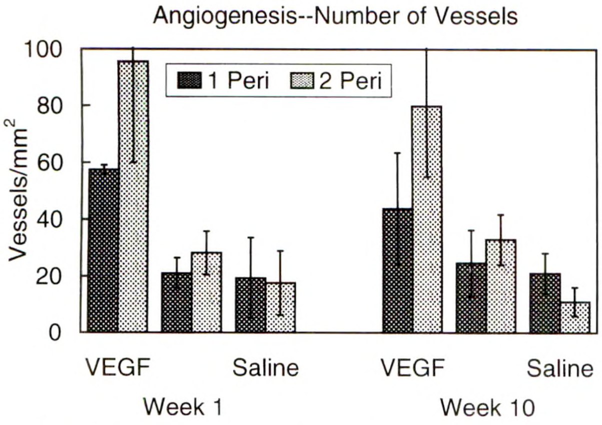

Fig 3. Angiogenesis on extraluminal surface of cartilage grafts, measured in vessels per square millimeter, at 1 and 10 weeks after operation (mean ± SD; $p < .001$ between VEGF and saline groups at both time periods). 1 Peri — single perichondrial grafts; 2 Peri — double perichondrial grafts.

as compared to 0.86 ± 0.76 mm^2 for those treated with AdCMVLacZ ($n = 5$) and 1.97 ± 1.10 mm^2 for the group treated with saline solution ($n = 5$; $p = .177$; Fig 5). The single perichondrial grafts treated with AdCMV-VEGF121 ($n = 5$) showed, at 10 weeks, 0.68 ± 0.74 mm^2 of viability, as compared to 1.01 ± 0.88 mm^2 for the grafts treated with AdCMVLacZ ($n = 5$) and 1.69 ± 0.83 mm^2 for the control group ($n = 5$; $p = .800$).

No statistical differences in viability were observed between the groups treated with AdCMV-VEGF121, AdCMVLacZ, and saline solution at 1 week or 10 weeks after operation ($p = .660$). Additionally, perichondrial preservation on both sides made no difference in graft survival in any of the groups at any time point ($p = .223$).

DISCUSSION

Reconstruction of the airway with free cartilaginous grafts remains a challenging task for the surgeon. Previous experimental studies have demonstrated that autologous cartilage grafts undergo progressive necrosis and resorption that may be related to ischemia of the free cartilage.[14,15] Despite meticulous surgical technique, the dynamic and progressive nature of graft healing can lead to cartilage shrinkage with remodeling — hence the need for revision surgery shortly after primary surgical intervention. We hypothesized that ex vivo induction of angiogenesis would promote graft cartilage survival.

The study clearly demonstrates increased angiogenesis in the animals receiving cartilage grafts treated with the VEGF121 adenoviral construct. Angiogenesis is assessed and analyzed primarily on the extraluminal side of the graft, because of the predominance of neovascularization on that side. Angiogenesis is observed as early as 1 week after operation. A statistically significant increase in angiogenesis is also observed at 10 weeks after operation. The expression of the adenoviral transgenes is typically cleared within 7 days, so the long-term influence of VEGF121 at 10 weeks suggests an angiogenic effect remaining long after clearance of the VEGF121 trans-

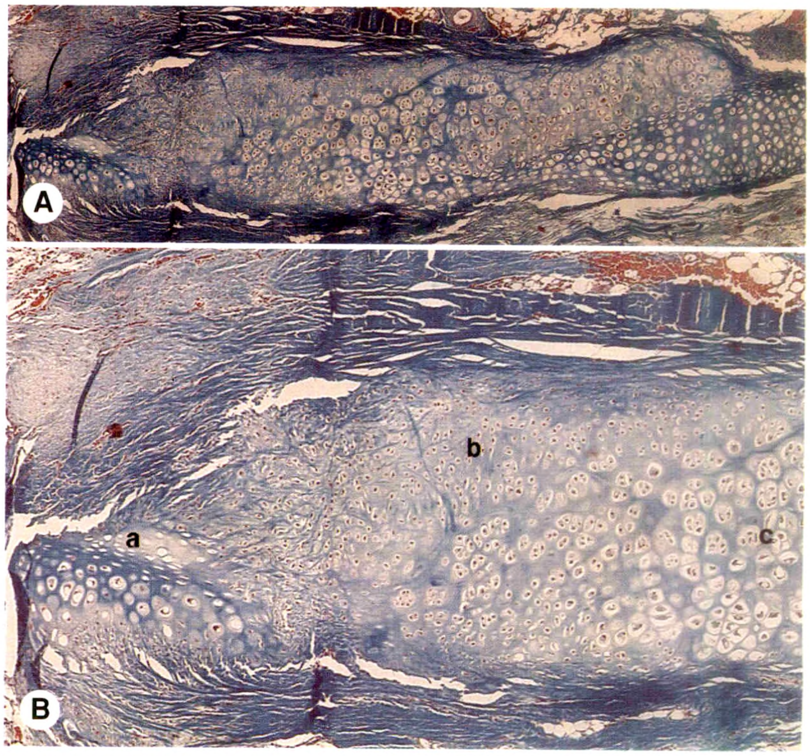

Fig 4. Montage of trichrome stain. **A**) Photomicrograph encompassing full length of graft (original ×100). **B**) Close-up view of same graft, demonstrating areas of necrosis (a), neochondrification (b), and viable cartilage (c; original ×400).

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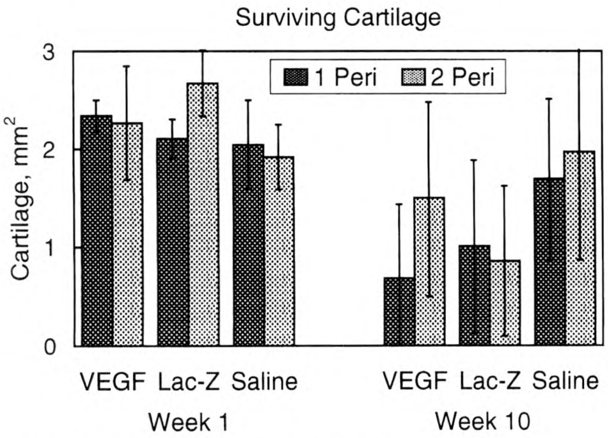

Fig 5. Cartilage survival, measured in square millimeters, at 1 and 10 weeks after operation (mean ± SD).

gene.[16] Although all of our reconstructions were performed with the perichondrium facing the airway lumen, the presence of the perichondrium on the extraluminal side of the graft further enhanced the re-formation of the fibrovascular network and angiogenesis on that surface. There was no significant difference in angiogenic pattern between the grafts treated with marker virus AdCMVLacZ and those treated with saline solution — a finding further emphasizing the lack of impact of the adenovirus itself in promoting angiogenesis. Additionally, there was no toxicity directly attributable to the virus itself, at either the early or late time points.

In terms of cartilage viability, there is progressive graft necrosis, resorption, and neochondrification at 10 weeks after operation. Graft survival is uniformly better in all specimens at 1 week versus 10 weeks after operation; this finding confirms the results of previous experiments.[14,17] Although treatment of the auricular grafts with AdCMV-VEGF121 or AdCMV-LacZ constructs had no detrimental effects, it did not enhance cartilage viability at 1 or 10 weeks after operation. Presence of perichondrium on the extraluminal side of the graft also made no difference in cartilage survival. Neochondrification was only demonstrated in the 10-week postoperative specimens, presumably because of the time required for cartilage remodeling and new cartilage formation to occur.[14] In contrast to our hypothesis, the angiogenic enhancement seen in specimens treated with AdCMV-VEGF121 did not translate to improved survival of the cartilage graft. This finding might be related to the choice of the angiogenic factor, the viral vector, the dose of the vector, or other experimental conditions. Alternatively, it might be necessary to induce a more ischemic state at the recipient site in order to realize the full benefit of the enhanced vascularity.

One of the limitations of this gene transfer strategy in an animal model with an intact immune system is the immune response generated by the adenovirus. Although AdCMVLacZ-treated grafts would not be expected to heal better, there was no detrimental effect of the virus. Additionally, the visibly enhanced neovascularization did not translate into improved graft survival. This finding calls into question the functional status of these vessels and their ability to establish timely revascularization of the ischemic perichondrium and deliver metabolites to the underlying cartilage. Last, our experiments used only a single angiogenic growth factor to enhance angiogenesis and cartilage viability. Although VEGF121 was demonstrated to induce an angiogenic effect, it was not able to alter the viability of the cartilage graft. Perhaps other VEGF isoforms or other angiogenic growth factors such as basic fibroblast growth factor would enhance graft survival.

Nevertheless, this novel study clearly demonstrates that regeneration of the fibrovascular network may be enhanced significantly with the use of an ex vivo strategy of adenovirus-mediated gene transfer of VEGF121 in laryngotracheal reconstruction. Further experiments are required to test other combinations of transfer vectors and angiogenic growth factors to maximize not just vessel formation, but cartilage graft survival and maintenance of luminal patency.

CONCLUSION

Adenovirus-mediated gene transfer of VEGF121 by ex vivo infection of cartilage used in airway reconstruction resulted in significant angiogenesis and neovascularization as compared to control specimens. However, the graft cartilage viability was not significantly enhanced by the virus or the VEGF transgene product. There was no significant toxicity or inflammation noted with the virus as compared to saline-treated controls. Although ex vivo gene transfer to cartilage with an angiogenic transgene may be a promising therapeutic strategy to enhance revascularization, future studies are required to optimize graft cartilage survival with angiogenic growth factors.

ACKNOWLEDGMENTS — Special thanks to Roger Marsh, PhD, Joanne Stow, RN, BSN, Bernard Martin, Huaqing Zhao, and Antoneta Radu for their technical expertise.

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GENOTYPIC AND PHENOTYPIC EXPRESSION OF VOCAL FOLD POLYPS AND REINKE'S EDEMA: A PRELIMINARY STUDY

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Although a great deal of research exists regarding lamina propria composition, no report exists that relates gene expression in benign laryngeal lesions to phenotypic markers. In this study, messenger RNA profiles for extracellular matrix proteins — procollagen I, collagenase, elastase, fibronectin, fibromodulin, decorin, hyaluronic acid synthase 2, and hyaluronidase — were completed on 5 polyps and 4 Reinke's edema specimens. These genotypic profiles were correlated to a videostroboscopic parameter of mucosal wave stiffness, which was used as a measurement of phenotypic expression. Polyps, characterized by stiffer mucosal waves, had higher levels of gene expression, whereas stiffer mucosal wave scores for Reinke's edema were associated with lower gene activity levels. This study supports the hypothesis that there is a relationship between genotypic expression found in polyps and Reinke's edema and phenotype as defined by a loss of or a decreased mucosal wave. The study also gives clues as to the proteins responsible for the phenotype.

KEY WORDS — decorin, extracellular matrix, fibromodulin, fibronectin, hyaluronic acid, hyaluronidase, larynx, metalloproteinase, mucosal wave, procollagen, stroboscopy, vocal fold.

INTRODUCTION

Vocal fold extracellular matrix (ECM) composition requires a constantly changing dynamic between cellular and matrix elements to maintain normal vibratory function. Matrix removal and synthesis occur simultaneously, and normal differentiation occurs when there is homeostasis between matrix formation and turnover. Regulation and homeostasis of the ECM membranous fold is poorly understood. We do not have a good idea of the mechanisms involved in turning on the genes that regulate the ECM, nor do we know what gene levels are altered when homeostasis is disturbed. In benign vocal fold disorders, there is likely a discrepancy between matrix turnover and synthesis that instigates vibratory dysfunction. The current study investigates gene expression levels in polyps and Reinke's edema. It provides insights into the biological changes that take place in these conditions and how these changes may be responsible for altered membranous vocal fold performance.

Extracellular matrix regulation involves proteins, ie, collagen and elastin, that are made by fibroblasts. Proteins are constantly being degraded and reproduced by enzymes (also made by fibroblasts) called matrix metalloproteinases (MMPs), ie, collagenases (MMP-1) and elastases (MMP-12). Last, there are tissue inhibitors of MMPs (TIMPs), which work to prevent matrix degradation by the MMPs. When there is homeostasis, there exists a tightly controlled balance among the proteins, MMPs, and TIMPs, producing a normal ECM. When the homeostasis becomes altered, there can be disruption in the expression of any of the three factors or any combination thereof. To date, no study of ECM regulatory mechanisms in normal vocal folds or vocal fold lesions has been undertaken. This lack may be due, in part, to difficulties in obtaining tissue samples. Ding and Gray[1,2] have investigated regulation of collagen and elastin in the vocal folds using a rat model.

Little is known about the ECM regulation of polyps or Reinke's edema. Select ultrastructural features have been histologically described. Decreased fibronectin, less basement zone injury, and increased vascular injury[3,4] characterize polyps, whereas increased fibrin, hemorrhage, and thickening of the basement membrane zone have been described in Reinke's edema.[5] Both lesions represent wound-healing paradigms. Inflammation and hemostasis-coagulation describe Reinke's edema and polyps, respectively. It has been hypothesized by Kotby et al[6] that these lesions represent a continuum of vocal fold injury and

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have essentially identical pathogeneses. Alterations in the histologic ultrastructural features provide insight so that one would expect precariousness in the ECM balance that would contribute to the anomalous mucosal wave that characterizes each lesion.

Altered gene expression in polyps and Reinke's edema may produce a phenotype that corresponds to their mucosal wave anomalies. The mucosal wave is visualized under stroboscopy and is an indirect measurement of the health of the body-cover lamina propria relationship.[7] Videostroboscopically, polyps are characterized by a stiffened, reduced mucosal wave,[7] whereas Reinke's edema is characterized by greater-than-normal movements of the mucosal wave.[8] A genotype produces various degrees of phenotypic expression. A genotype-phenotype association that correlates to an altered mucosal wave may help to explain the wide range of stiffness or pliability seen clinically among lesions.

The goal of the present study was to investigate ECM gene expression in vocal fold polyps and Reinke's edema and relate the gene expression to mucosal wave properties. The proteins of interest selected for this investigation have been described as normal components of the ECM of the vocal folds and are keenly involved in the wound healing cascade. These include collagen,[9-11] collagenase,[1] elastase,[2] decorin,[12] fibronectin,[12] fibromodulin,[12] hyaluronic acid synthase 2,[13] and hyaluronidase.[13] To carry out the above-stated goal, several hypotheses were considered. First, it is believed that there will be greater gene expression levels for vocal fold polyps than for Reinke's edema. Previous researchers have described polyps as representing an acute insult,[3,6] implying a wound repair response that is larger and shorter in duration. Second, correlated to the mucosal wave, the greater gene expression activity in vocal fold polyps will be associated with a stiffer phenotype. Wound healing is associated with increased biological activity; therefore, greater gene expression of the selected proteins in either benign disorder would likely be associated with more aggravated wound healing activity.

METHODS

Sample Attainment. Five vocal fold polyps and 4 Reinke's edema samples were taken from specimens obtained from patients during surgical excision of the lesions. The tissue samples were immediately placed in RNAlater (Ambion, Inc, Austin, Texas). After 24 hours at 4°C, the tissue was snap-frozen in liquid nitrogen and stored until extraction. The processing of the tissues did not alter the microphonosurgical procedures. The University of Wisconsin–Madison Health Sciences Human Subjects and University of Utah Institutional Review Board committees approved the protocol for obtainment of tissue samples.

Extraction of Total RNA. Total RNA was isolated from tissue samples by the guanidine isothiocyanate–phenol method.[14] The tissue was homogenized (PRO 200, Intermountain Scientific Co, Kaysville, Utah) and lysed in Trizol reagent (Gibco-BRL, Life Technologies, Grand Island, New York). Samples were extracted by means of isoamyl alcohol–chloroform extraction and isopropanol precipitated at room temperature for 10 minutes. After an ethanol wash, total RNA was yielded and resuspended in RNA Storage Solution (Ambion, Inc). The total RNA concentration was quantified with spectrophotometric analysis using the formula 1 optic diameter at 260 nm equals 40 µg RNA per milliliter. RNA purity and quality was assessed by Northern analysis, and 3 µg of resuspended RNA was run on a 1.2% agarose gel containing 6.7% formaldehyde.

Reverse Transcriptase–Polymerase Chain Reaction Amplification. One microgram aliquot of total RNA was treated with 5 units of RNase-free DNase I (Amersham Pharmacia, Piscataway, New Jersey) for 20 minutes at 37°C, followed by inactivation of the DNase I at 75°C, to eliminate genomic DNA contamination. The First Strand cDNA Synthesis Kit (Boehringer Mannheim, Indianapolis, Indiana) was used for reverse transcription. Reverse transcription was carried out for 95 minutes at 42°C in a volume of 20 µL containing 1 µg of DNase I–treated RNA, 20 mmol/L of deoxynucleoside 5'-triphosphate, 1.6 µg of Oligo-p(dT)15 primer, 50 units of Rnase inhibitor, 5 mmol/L magnesium chloride, and 25 units of avian myeloblastosis virus reverse transcriptase in 1× reverse transcription buffer. Complementary DNA (cDNA) was stored in a volume of 100 µL at –80°C.

The oligonucleotide primer set sequences, polymerase chain reaction (PCR) product sizes, and optimal conditions of primer sets used are listed in Table 1. Using gene sequences published in Genbank and the program Primer3,[15] we designed primer set sequences. The DNA/Peptide Facility at the Huntsman Cancer Institute at the University of Utah completed the synthesis of sequences. Under optimal conditions, all primers were verified to produce only one single PCR band at the expected size. The correct sequences of PCR products were verified by DNA sequencing.

The PCR reaction mixture consisted of (final volume of 25 µL) 10 mmol/L Tris–hydrochloric acid, 50 mmol/L potassium chloride (pH 8.3), 1.5 mmol/L magnesium chloride (except hyaluronic acid syn-

TABLE 1. SEQUENCES OF POLYMERASE CHAIN REACTION PRIMERS, LENGTHS OF POLYMERASE CHAIN REACTION PRODUCTS, AND OPTIMAL CONDITIONS

	Primers (5′-3′)			*Magnesium*	
	Forward	*Reverse*	*Product (bp)*	*Chloride (mmol/L)*	*AT (°C)*
Procollagen 1	GTTGGTGCTAAGGGTGAAGC	ACCAGTGTCTCCTTTGCTGC	294	1.5	56
Collagenase (MMP-1)	CAACTTACATCGGTTGCGG	ACCGGACTTCATCTCTGTCG	395	1.5	50
Elastase (MMP-12)	CTGCTGATGACATACGTGGC	GTTTGGGATAACCAGGGTCC	498	1.5	62
Hyaluronidase	TGGGGCTTCTACCTCTTTCC	TGAGGTACTGGCAGGTCTCC	421	1.5	62
Hyaluronic acid synthase 2	GTTGGCTACCAGTTTATCCAAACG	CTTTATGTGACTCATCTGTCTCAC	403	2	56
Fibromodulin	CACTTTCCTCCACAGATGCC	GCTGCTTTCTCAACTCAGCC	387	1.5	62
Decorin	GATGCAGCTAGCCTGAAAGG	TCACACCCGAATAAGAAGCC	274	1.5	50
Fibronectin	ACCAACCTACGGATGACTCG	CATCATCGTAACACGTTGCC	300	1.5	56
GAPDH	ACCCAGAAGACTGTGGATGG	TGAGCTTGACAAAGTGGTCG	382	1.5	56

AT — annealing temperature, GAPDH — glyceraldehyde-3-phosphate dehydrogenase.

thase 2, which used 2 mmol/L), 0.2 mmol/L of each deoxynucleoside 5′-triphosphate, 0.5 µmol/L primers 5′ and 3′ respectively, 1 unit of Taq polymerase mixed with an equal volume of TaqStart antibody (Clontech Lab, Inc, Palo Alto, California), and various amounts of cDNA equivalent to 1.25 to 20 ng of total RNA. The volume of cDNA used in each reaction was calculated through an optimization step to avoid saturation caused by excess cDNA. Simultaneous amplification (35 cycles) of twofold serial dilutions of the cDNA was completed, and the densities of the PCR products were plotted against the volumes of the starting cDNA. The cDNA amount chosen was based upon the PCR product that falls within a linear range before it reaches maximum plateau. Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with all amplifications provided a comparative quantification of PCR products. All amplifications were completed in duplicate to verify validity and reliability. A Peltier Thermal Cycler PTC 2000 (MJ Research, Watertown, Massachusetts) was used. Amplification was carried out for 35 cycles. After an initial denaturation at 94°C for 3 minutes, each cycle consisted of denaturing at 94°C for 30 seconds, annealing at 50°C to 62°C (Table 1) for 30 seconds, and elongation at 72°C for 30 seconds, followed by 7 minutes of extra elongation.

A 2% agarose gel with 0.5 µg/mL of ethidium bromide was used for electrophoresis of the PCR products. Images were captured and analyzed with Gel-Expert (Nucleotech Corp, San Mateo, California). To determine the level of transcript with standardized densitometry values, the ratio of target messenger RNA (mRNA) to the housekeeping gene GAPDH was ascertained.

The median arbitrary densitometry values were calculated for the vocal fold polyp and Reinke's edema groups. Individual mRNA scores for each protein were given a +1 if they were greater than the median value and −1 if they were lower than the median value. A total gene expression score (TGES) was tabulated for each sample.

Stroboscopic Analysis. Preoperative stroboscopic evaluations were completed as part of the patients' routine physician visit. Videostroboscopic S-VHS tapes were produced on the Kay Elemetrics Rhino-Laryngeal Stroboscope 9100 (Lincoln Park, New Jersey) and rated by 5 experienced speech-language pathologists (mean years of voice experience, 14) from the University of Wisconsin–Madison Hospital and Clinics. As an indicator of stiffness, the mucosal wave was rated on a 6-point scale (0 = normal, 1 = slightly decreased, 2 = moderately decreased, 3 = severely decreased, 4 = barely perceptible, and 5 = absent) taken from the University of Wisconsin Stroboscopic Assessment of Voice.[16] An average strobe stiffness score was determined for each sample. Intrarater reliability was performed on all samples. All measures yielded nonsignificant differences ($p = .124$) for rating with a paired *t*-test.

Western Blot. Western blotting was completed for collagen I and fibronectin for the 5 vocal polyp samples. After RNA and DNA were removed (as above), proteins were precipitated with isopropyl alcohol, washed in 0.3 mol/L guanidine hydrochloride in 95% ethanol 3 times, submitted to a final wash in ethanol, and dissolved in 1% sodium dodecyl sulfate. The concentration of proteins was determined with the DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, California). Ten micrograms of protein from each sample was separated with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (7.5% for fibronectin and elastin; 6% for collagen I) under reducing conditions. The proteins were then transferred

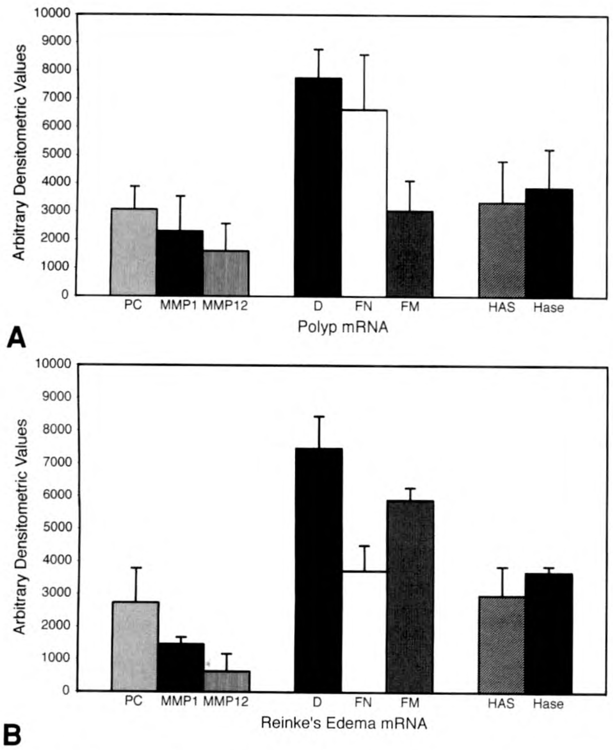

Fig 1. Expression of genes coding procollagen (PC), collagenase (MMP-1), elastase (MMP-12), decorin (D), fibronectin (FN), fibromodulin (FM), hyaluronic acid synthase 2 (HAS), and hyaluronidase (Hase) in **A**) vocal fold polyps and **B**) Reinke's edema as determined by reverse transcriptase–polymerase chain reaction. Expression levels were normalized with respective glyceraldehyde-3-phosphate dehydrogenase messenger RNA (mRNA) levels.

onto polyvinylidene difluoride membrane (Millipore Corp, Bedford, Massachusetts). The membranes were blocked in 5% milk powder in Tris-buffer saline solution containing 0.05% Tween (TBS-T) overnight at 4°C, then incubated with mouse anti–cellular fibronectin monoclonal antibody (Chemicon International, Inc, Temecula, California) or rabbit anti-mouse collagen type I antibody (Research Diagnostics, Inc, Flanders, New Jersey) for 2 hours at room temperature. After extensive washing with TBS-T, the membrane was reacted with the secondary goat anti-mouse or goat anti-rabbit antibody conjugated with horseradish peroxidase. Chemiluminescent reaction with the ECL Western Blotting Analysis System (Amersham Pharmacia) was used to detect protein bands by means of autoradiography, exposing the membrane to Fuji RX film. Blots were quantified by measurement of extended chemiluminescence in GelExpert (Nucleotech Corp).

RESULTS

Analysis of mRNA. The average levels of mRNA expression, normalized with respect to GAPDH, for 5 vocal fold polyps and 4 Reinke's edema samples are presented in Fig 1. Structural variations group ECM constituents: fibrous proteins (collagen, MMP-1, MMP-12), interstitial proteoglycans (decorin, fibronectin, fibromodulin), and glycosaminoglycans (hyaluronic acid synthase 2, hyaluronidase).

For the fibrous proteins — precursor procollagen I (measure of novel collagen I) and the MMPs collagenase and elastase — similar patterns of expression were ascertained for polyps and Reinke's edema. The MMP-1 and MMP-12 densitometric values were greater for the Reinke's edema group than for the polyp group. It must be confirmed whether the mRNA levels found here reflect protein levels.

The expression levels of decorin, fibronectin, and fibromodulin represent the interstitial and small leucine-rich proteoglycans. Large mRNA levels of decorin with decreasing mRNA levels of fibronectin and fibromodulin were observed in both disorders. The relationship trend among the three proteoglycans differed between polyps and Reinke's edema. Fibronectin is decreased in Reinke's edema as opposed to polyps, whereas polyps show a reduction in fibromodulin to a greater extent than does Reinke's edema.

The hyaluronic acid synthase 2 and hyaluronidase expression patterns of mRNA levels were similar across disorders. The presence of both the genes hyaluronic acid synthase 2 and hyaluronidase — one that breaks down hyaluronic acid and one that makes hyaluronic acid — suggests active turnover and remodeling.

Stroboscopic Analysis and Total Gene Expression Scoring. The means and standard deviations for mucosal wave scores are summarized in Table 2.[16] The scores represent a wide range of stiffness and pliabil-

TABLE 2. MUCOSAL WAVE SCORES AND TOTAL GENE EXPRESSION SCORES

	Polyp Specimens					*Reinke's Edema Specimens*			
	1	*2*	*3*	*4*	*5*	*1*	*2*	*3*	*4*
Mucosal wave score (mean ± SD)*	5 ± 0	2.75 ± 0.5	5 ± 0	4 ± 0.5	3.25 ± 0.5	1.25 ± 0.5	1.25 ± 0.5	5 ± 0	5 ± 0
Total gene expression score†	4	–6	4	2	–4	4	2	–2	–4

*From Koschkee and Rammage.[16] 0 = normal, 1 = slightly decreased, 2 = moderately decreased, 3 = severely decreased, 4 = barely perceptible, 5 = absent.

†From median arbitrary densitometry values. Individual messenger RNA scores for each protein were given +1 if greater than median and –1 if lower than median.

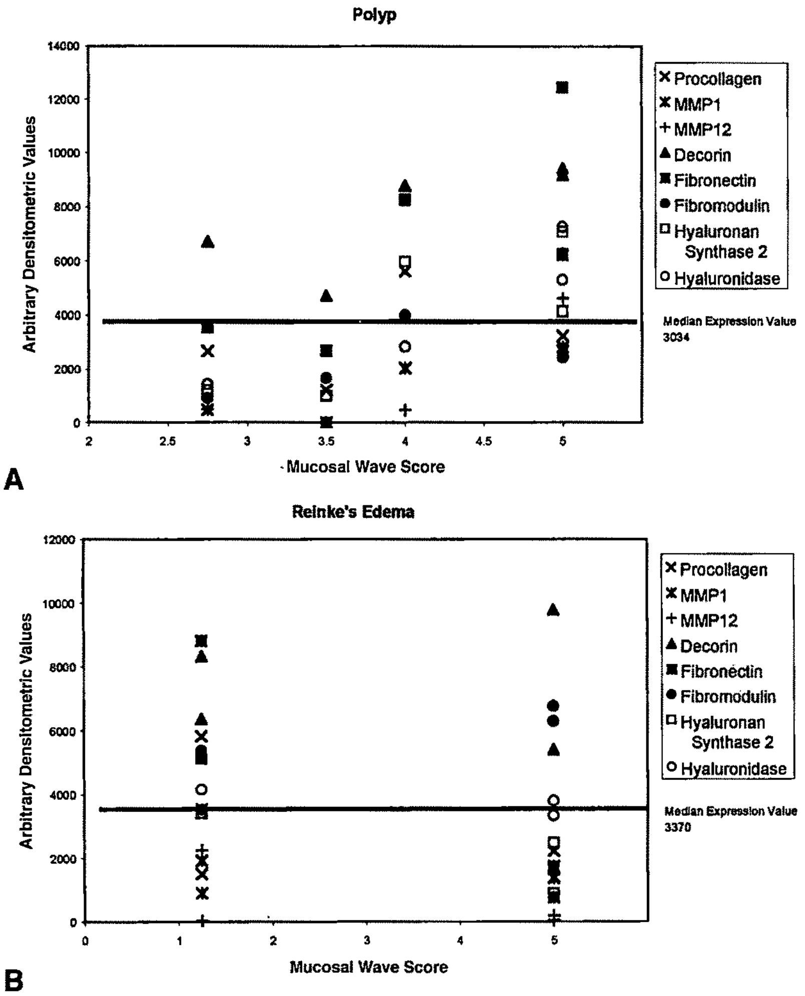

Fig 2. Expression of gene coding procollagen, collagenase (MMP-1), elastase (MMP-12), decorin, fibronectin, fibromodulin, hyaluronic acid synthase, and hyaluronidase in **A)** vocal fold polyps and **B)** Reinke's edema as function of mucosal wave score. Median expression value for polyps of 3,034 is represented by horizontal dark bar in A. Median expression value for Reinke's edema of 3,370 is represented by horizontal dark bar in B. Protein groups above were scored positive, and those below, negative, to calculate total gene expression score (TGES). Positive TGES indicates higher levels of gene activity; negative TGES indicates lower levels of gene activity.

ity. The vocal fold polyp scores range from 2.75 to 5 (severely decreased to absent mucosal wave). The Reinke's edema scores range from slightly decreased (1.25) to absent (5).

The TGESs that were calculated on the basis of the median gene expression scores of 3,034 and 3,370 for polyps and Reinke's edema, respectively, are shown in Table 2. The more positive the TGES score is, the higher the gene expression levels are above the median; the more negative TGES score, the lower the gene expression levels are below the median. There was an inverse relationship between the two pathological conditions in terms of how TGESs related to mucosal wave scores. Polyps whose mucosal waves were rated as either a 4 or a 5, indicating a barely perceptible or absent wave, corresponded to positive TGES. In other words, polyps with lower stiffness scores received negative TGES scores, indicating that the stiffer the mucosal wave, the greater the level of gene activity. Figure 2A demonstrates individual polyp protein mRNA expression levels as a function of mucosal wave score. Conversely, mucosal wave scores for Reinke's edema lesions were 1.25 (slightly decreased) or 5 (absent; Fig 2B). Table 2 demonstrates that slightly decreased mucosal wave scores corresponded to a positive TGES, whereas a negative TGES corresponded to absent mucosal wave scores. This proportional relationship can be visualized in Fig 2B, in which the stiffer mucosal wave scores correspond to lower gene expression activity levels, and the slightly decreased mucosal wave scores correspond to higher gene expression activity levels.

Western Blots. To determine whether protein levels were similarly expressed in polyps, we performed analysis by Western blotting with anti-collagen and anti-fibronectin antiserum (Fig 3). Single bands at approximately 65 kd and 115 kd, consistent with the predicted masses of the proteins fibronectin and collagen, respectively, were detected for all polyp samples. For both proteins, there were a range of densitometric values; however, the values for collagen were considerably smaller (<1,000) than those for fibronectin (>2,500). Although the sample size was small, several trends were evident. The smallest collagen quantities were associated with the polyps that had the lowest mucosal wave scores. A reciprocal rela-

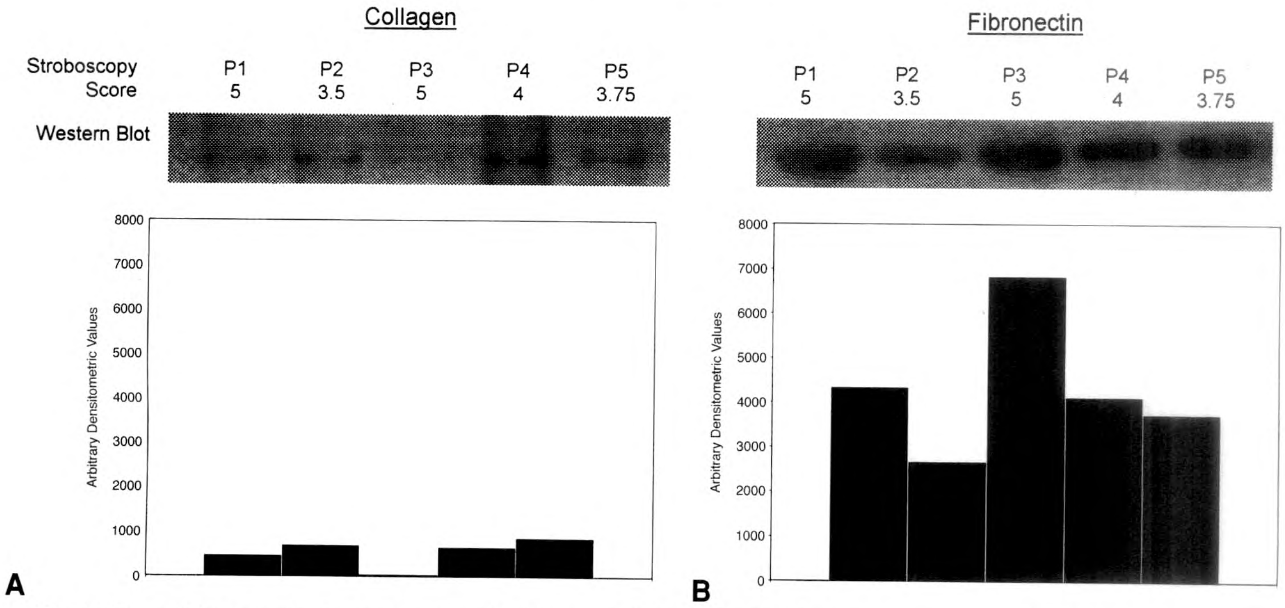

Fig 3. Western blot analysis of **A)** collagen and **B)** fibronectin in 5 vocal fold polyp samples, P1 through P5. Mean stroboscopy scores indicate levels of stiffness (0 = normal, 1 = slightly decreased, 2 = moderately decreased, 3 = severely decreased, 4 = barely perceptible, and 5 = absent). Bar graphs are results of densitometric analysis of individual polyp protein levels for collagen and fibronectin.

tionship was demonstrated for fibronectin, in that the highest fibronectin quantities were associated with polyps with absent mucosal wave scores.

DISCUSSION

Advances in protein and genetic engineering allow for the possibility of expanding our knowledge of the ECM, particularly regarding its role in providing homeostasis. On the basis of our knowledge of the vocal fold cover, it is presumed that pathophysiological changes of the vocal fold ECM produce disease states, such as polyps and Reinke's edema, that alter normal vibratory function and create debilitating dysphonias. This study has characterized gene expression levels for a subset of ECM proteins and demonstrated that the level of gene expression activity is conceivably related directly to mucosal wave stiffness.

The ECM proteins that provide homeostasis arise from the fibroblast. Measurement of mRNA levels as a means of gene transcription profiling provides insight into pretranslational ECM regulation. In this study, 3 major trends in similarity in mRNA levels for polyps and Reinke's edema were observed. First, the gene for procollagen I expression was upregulated. (Procollagen I is a marker for new collagen production.) This was not surprising, as previous research of collagen protein levels during wound healing[17] demonstrated that the rate of new collagen synthesis is high in the early stages of wound repair. Nevertheless, given that vocal trauma caused by repetitive vibratory forces can be considered an etiologic contributor to both polyps and Reinke's edema, and that vocal trauma is most often consistent, it could be hypothesized that neither of these lesions are able to progress to later stages of wound repair, and that high levels of new collagen are represented. Second, MMP-12 and MMP-1 were downregulated compared to procollagen I. The MMP-12 functions as an enzyme breaking down elastin, a protein that is present in ECM and in low levels in wounded tissue.[18] It is difficult to conjecture, without elastin transcription levels, whether less mRNA elastin is synthesized or whether MMP-12 is responsible for the breakdown of normal elastin levels. MMP-1 is usually present after injury to provide constant enzyme degradation and remodeling.[17] Third, analogous transcriptional trends for hyaluronic acid synthase 2 and hyaluronidase were seen in both disorders, suggesting active remodeling at the protein level. Hyaluronic acid is a complex glycosaminoglycan that is made by hyaluronic acid synthase 2 and is broken down by hyaluronidase. It is biologically active and is thought to play an important role in wound repair. The absence of hyaluronic acid has been implicated in scar development.[19,20] Additionally, the contribution of hyaluronic acid to maintaining normal viscoelastic properties of the vocal folds has been reported.[11,21] A decrease in hyaluronic acid in the lesions might have several implications, including the inability of the ECM to adequately repair itself, and it might contribute to altered tissue viscoelastic properties. This possibility provides further support to the notion that continued vocal abuse prevents the final stages of wound healing.

Interstitial proteins are believed to play a major role in chemical signaling between cells and serve to regulate the activity of other proteins. Differences in transcriptional trends between the two pathological groups provide interesting insight into potential etiologic differentiation. High levels of decorin mRNA were present in both disorders. It is possible that high levels of decorin are present to regulate other proteins. For example, decorin is able to bind and neutralize transforming growth factor beta (TGF-β) through its core protein.[22] TGF-β is released during wound healing by either degranulating platelets or cells, and it has been implicated in excess ECM deposition.[23] Uncontrolled expression of TGF-β can have pathological consequences.

Differences in regulation of fibronectin and fibromodulin transcription levels between the two pathological groups may provide insight into differences in mucosal wave characteristics. Fibronectin transcription levels are clearly downregulated in Reinke's edema as compared to polyps. The ability of fibronectin to induce cell migration and ECM synthesis suggests that its persistence might be involved in the development of fibrosis, which might be directly related to mucosal wave viscoelastic parameters. The decreased levels of fibronectin in Reinke's edema suggest that there is activity toward a state of less stiffness. Fibromodulin plays a role in collagen fibrillogenesis. Fibromodulin-null animals have collagen fiber bundles that are disorganized and have abnormal ECM morphological characteristics.[24] Fibromodulin is downregulated in dermal scar[25] and can bind and potentially inhibit TGF-β. A downregulation of fibromodulin, as found in the polyp lesions, suggests a disorganized ECM regulation that may be moving toward a stiffer environment. A disorganized ECM with increased stiffness and decreased viscosity has been found in vocal fold scarring,[26] a condition that represents poor wound repair reconstitution of tissue matrix. Both increases in fibronectin and decreases in fibromodulin support a stiffer phenotype in polyps.

The TGESs in Table 2 and Figs 3 and 4, in conjunction with the transcriptional levels of mRNA, provide insight into the etiologic pathogenesis of these two lesions. These lesions appear to have very different overall gene activity levels that characterize their individual wound healing paradigms. Polyps are more often regarded as acute, focal injuries, whereas Reinke's edema is regarded as a diffuse, chronic condition. It is difficult to postulate whether there is a disorder in the levels of fibrous proteins, MMPs, or TIMPs, but higher activity levels would indicate a system that is undergoing remodeling because of protein turnover. Integrating our findings for polyps of upregulated fibronectin and downregulated fibromodulin mRNA levels and higher TGESs seems to corroborate our hypothesis. In Reinke's edema, lower TGESs are associated with a stiffer phenotype, indicating less gene activity. This converse relationship between TGES and gene activity level, combined with decreased fibronectin and increased fibromodulin, indicates a regulation pattern that is distinctly different from that of polyps — one that is tipped toward a state of less stiffness.

Transcription levels were confirmed with measurement of protein translation levels for collagen and fibronectin for the polyp group. The changes in the protein levels parallel those found in mRNA levels and TGES-stroboscopy analysis. Low collagen levels appear to be related to a stiffer phenotype, consistent with a report that vocal fold scars have less collagen and increased stiffness.[26] High fibronectin protein levels allied with stiffer mucosal wave scores corroborate the hypothesis of fibronectin inducing ECM synthesis,[27] which can produce an unorganized matrix. Fibronectin has an adhesive character, attracting cells and macromolecules[27] and altering molecular configurations, which might directly affect mucosal wave stiffness. Western blot analysis needs to be completed for the remainder of the proteins evaluated, to confirm our hypotheses.

Even though our data present provocative findings, they are not without restrictions. The sample size is small and may represent a small fraction of the biological properties of polyps and Reinke's edema. Clinically, there are a large variety of lesion types that encompass both of these groups. Further study of a larger number of lesions, as well as within group categories, warrants attention. Additionally, because all of the lesions studied came from different persons, environmental influences on genotypic and phenotypic expression cannot be ruled out. To the extent possible, future studies should integrate environmental factors.

CONCLUSIONS

Transcription-level profiles of ECM proteins, TGES, and Western blot analysis of vocal fold polyps and Reinke's edema indicate that these lesions may have differing biological foundations. Rather then representing a continuum, polyps and Reinke's edema appear to have differing ECM regulation that is probably responsible for their differing phenotype. Two interstitial proteoglycans — fibromodulin and fibronectin — provide particular support for this hypothesis. These findings suggest that as we learn more about ECM regulation, we may be able to determine better diagnostic and management strategies.

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TRANSTRACHEAL OXYGEN CATHETERS IN A PEDIATRIC POPULATION

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This is the first report to evaluate transtracheal oxygen catheter (TTOC) use in a pediatric patient series. Seven pediatric patients (4 boys and 3 girls) received TTOCs in 2 tertiary care medical centers. The medical indications included bronchopulmonary dysplasia in 4 patients and tracheomalacia in the other 3. The average age at the time of placement was 22 months (range, 2 weeks to 37 months). Catheter placement for 4 patients was through an open tracheotomy stoma. In 3, placement was through a percutaneous technique. The follow-up ranged from 2 weeks to 5 years. There were no long-term complications. Transient needs for supplemental oxygen were all met by the TTOC system. In 4 patients, the catheter has been removed because of resolution of the supplemental oxygen requirements. Minor complications included skin site infection and mucus plugging. In 1 patient, accidental dislodging of the catheter led to its replacement in the operating room. In 1 percutaneous placement, a pneumothorax occurred and resolved without any persistent morbidity. We conclude that transtracheal oxygen delivery can be a reasonable alternative to a nasal cannula or formal tracheotomy in selected pediatric patients in whom long-term oxygen delivery, but not an alternate airway, is required. In order to avoid complications, meticulous technique must be adhered to in using the percutaneous approach for placement.

KEY WORDS — oxygen delivery, pediatrics, transtracheal oxygen catheter.

INTRODUCTION

In adult populations, transtracheal oxygen catheters (TTOCs) have been proposed as an effective mechanism of long-term oxygen delivery. They offer distinct advantages over more traditional methods of oxygen supplementation, such as modified or conventional nasal cannulas. The side effects of nasal crusting, nasal obstruction, dry throat, allergic reactions, and epistaxis are not seen with TTOCs.[1] Other reported benefits of TTOCs include improved exercise tolerance and reduction of breathlessness,[2,3] decreased rate of hospitalization,[3] enhanced patient adherence,[3,4] and a reduction of required oxygen flow rate.[3-6] Furthermore, the discomfort, inconvenience, and social stigmata of nasal oxygen cannulas would seem to make TTOCs more attractive to patients. Because of the disadvantages related to nasal cannula use, in children it is not uncommon to use a formal tracheotomy tube as a means of providing oxygen supplementation, even when the tube is no longer needed as an airway. Although some of the drawbacks of nasal oxygen delivery are probably magnified in children, there is only 1 reported case of TTOC use in a pediatric patient.[7] This low incidence of clinical use of TTOCs in children may be related to concerns about possible complications attributable to the insertion and usage of these catheters. In adults, minor problems related to TTOCs (such as mucus plugging and cough) are common, but severe complications (such as pneumothorax and pneumomediastinum) are uncommon.[8] In order to determine the usefulness, efficacy, and safety of TTOCs in children, we prospectively followed a cohort of 7 pediatric patients in whom TTOCs were used as an alternative to a nasal cannula or formal tracheotomy.

METHODS

We followed a series of 7 pediatric patients who underwent placement of a TTOC as a primary mode of oxygen supplementation. The patients were followed for length of placement, adequacy of oxygen supplementation, complications, and reason (if any) for removal.

In our series, 3 patients underwent percutaneous placement of the TTOC. The placement is done under bronchoscopic guidance. Direct visualization of the catheter in the trachea helps prevent potentially life-threatening placement complications such as pneumothorax, pneumomediastinum, and posterior tracheal wall perforation.

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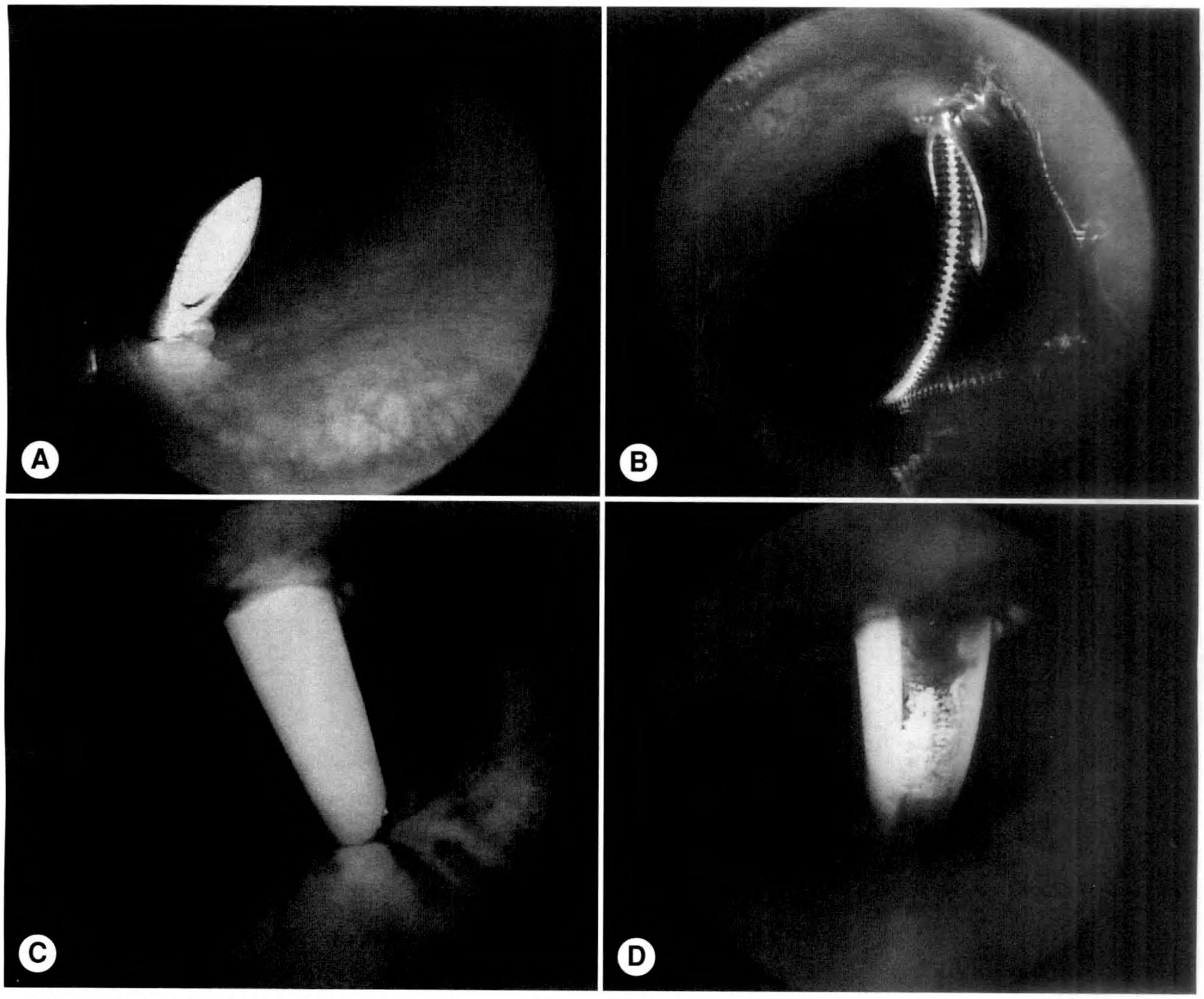

Fig 1. Bronchoscopic view of percutaneous placement of transtracheal oxygen catheter (TTOC). **A**) 19-Gauge needle is inserted percutaneously into tracheal lumen. **B**) Soft guide wire is inserted through needle. **C**) Dilator is passed over wire. **D**) After removal of dilator, TTOC is inserted over wire.

The patient is brought into the operating room, and general anesthesia is induced. The patient's head is extended, and the anterior neck is sterilely prepared and draped. The thyroid cartilage, cricoid cartilage, and suprasternal notch are identified. Standard rigid bronchoscopy is performed with the assistance of a rigid telescope passed through the bronchoscope's lumen. The patient is ventilated through the bronchoscope.

A percutaneous placement technique is used to place the TTOC. First, a 19-gauge needle is inserted approximately 1 cm below the cricoid cartilage, directly in the midline of the neck, into the trachea. An assistant looking through the bronchoscope confirms the location of the needle. Care is taken not to perforate the posterior wall. A soft guide-wire is passed through the needle into the tracheal lumen. The needle is removed, leaving the wire in place. A dilator is passed over the wire to enlarge the tract. The dilator is removed, leaving the wire in place. Finally, the TTOC is passed over the wire, into the tracheal lumen. The wire is removed, and the TTOC is secured externally in place. A chest radiograph is obtained in the recovery room to rule out pneumothorax or pneumomediastinum.

Figure 1 depicts the procedure from the bronchoscopic viewpoint. Figure 2 shows a standard TTOC. Note that the shorter end of the catheter measures 5 cm. This is the end that is placed into the trachea.

RESULTS

Four boys and 3 girls were included. The average age at placement was 22 months (range, 2 weeks to 37 months). The medical indications for oxygen requirement and TTOC included bronchopulmonary dysplasia (BPD) in 4 patients and tracheomalacia in 3. The method of catheter placement in 4 patients was through an open tracheotomy stoma when the

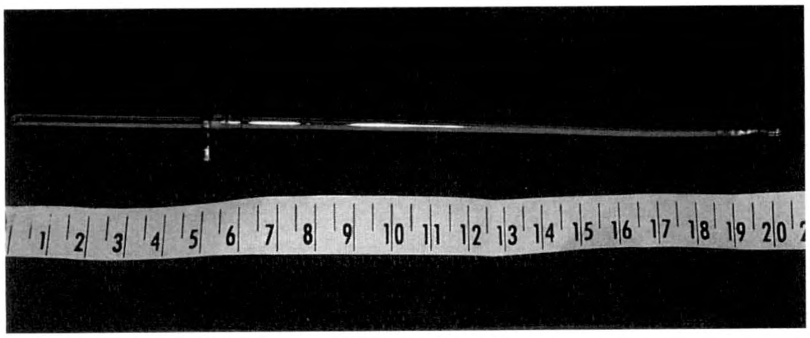

Fig 2. Photograph of standard pediatric TTOC. Shorter end measures 5 cm and is placed in trachea. Longer end is external, allowing for connection to standard oxygen catheter tubing. Rule is in centimeters.

cannula was no longer needed as an airway, but oxygen supplementation was still required. In the other 3 patients, the TTOC was placed by the percutaneous technique under bronchoscopic guidance. The follow-up ranged from 1 month to 5 years. Oxygen requirements (defined as the maintenance of an oxygen saturation of at least 94%, with flow rates of 1 to 2 L/min of oxygen) were met in all cases. There were no long-term complications. In 1 percutaneous placement case, a pneumothorax developed, requiring a chest tube for 2 days. It resolved without any persistent morbidity. Minor complications included mucus plugging in 2 cases, chronic cough in 1 case, and local skin infection in 1 case. Accidental dislodgment occurred in 1 case, requiring replacement of the TTOC in the operating room. In 4 patients, the catheter has been removed because the need for supplemental oxygen resolved. Three continue to use the TTOC. These results are summarized in Tables 1 and 2.

DISCUSSION

Although the safety and efficacy of TTOCs have been established in adults, their use has not been documented in a pediatric patient series. Although the TTOC is generally regarded as safe in adults,[8-10] the rate of complications related to its placement can be high. Rates as high as 30% for early complications and 73% for late complications have been reported.[8] Most of the early complications are related to the placement of the catheter and to maintenance of the immature, healing tract site.[5,11] In 4 of our patients, a mature tract into the tracheal lumen already existed from a previous tracheotomy, eliminating the complications related to the immature site. Among the other 3 patients, 2 had complications related to the insertion of the catheter. In 1, a chest tube was required for 2 days because of a pneumothorax, which occurred despite the use of a bronchoscope to guide placement. In the other case, the catheter was dislodged hours after placement, requiring a return trip to the operating room to confirm the replacement of the TTOC.

Mucus plugging seems to be the most common late complication related to TTOCs. It is caused by high oxygen flow rates resulting in drying of tracheal secretions and crusting around the catheter tip. The catheter must be removed, stripped, and cleaned periodically. The plugging can cause obstruction of the TTOC lumen. In a worst-case scenario, the mucus plug can progress to tracheal lumen obstruction and

TABLE 1. SUMMARY OF CASES

Patient	*Sex*	*Age at Placement*	*Diagnosis*	*Method*	*Duration*	*Removal*	*Complications*
1	F	7 y	BPD	Percutaneous, Nov 1993	3 mo	Received lung transplant	Infection at site
2	M	18 mo	Tracheomalacia, GERD	Percutaneous, Jan 1998	26 mo	Not yet removed	Pneumothorax
3	F	30 mo	Tracheomalacia, VATER	Percutaneous, Apr 1998	23 mo	Not yet removed	Dislodgment
4	M	5 y, 6 mo	BPD	Previous tracheotomy site, June 1997	32 mo	Not yet removed	None
5	F	3 y, 10 mo	BPD	Previous tracheotomy site, June 1993	36 mo	Decreased oxygen requirement	Cough, mucus plugging
6	M	6 y, 3 mo	BPD	Previous tracheotomy site, Aug 1996	36 mo	Decreased oxygen requirement	Mucus plugging
7	M	2 y, 4 mo	Tracheomalacia, DiGeorge syndrome	Previous tracheotomy site, July 1998	2 wk	Decreased oxygen requirement	None

BPD — bronchopulmonary dysplasia, GERD — gastroesophageal reflux disease, VATER — *v*ertebral defects, imperforate *a*nus, *t*racheo*e*sophageal fistula, and *r*adial and *r*enal dysplasia.

TABLE 2. SUMMARY OF COMPLICATIONS

Complications	*No. of Patients*
Minor	
Mucus plugging	2/7
Infection at site	1/7
Cough	1/7
Major	
Pneumothorax	1/7
Dislodgment	1/7

Minor complications occurred late, and major complications occurred early.

acute respiratory distress.[12] It is crucial to keep adequate humidification attached to the oxygen source at all times. In our series, 2 of 7 patients had recurring problems with mucus balls that required frequent cleansing and stripping. In both cases, lower respiratory tract infections seemed to contribute. No serious complications from the mucus crusts have occurred.

The reasons for oxygen requirement in our series included BPD and tracheomalacia. The patient's oxygen needs were met in all cases. Oxygen saturations of at least 94% were maintained on low oxygen flow rates. It is important to note that more than half of our patients no longer require the use of a TTOC, as they no longer need oxygen supplementation. This finding is in contradistinction to adults with chronic obstructive respiratory diseases, who may require long-term (or permanent) oxygen replacement. In children, it is likely that the oxygen need will decrease over time to the extent that the TTOC can be removed.

The advantages attributable to TTOCs may have a greater impact in children than in adults. First, transtracheal oxygen delivery has been established as more effective than nasal delivery.[2,13] By decreasing anatomic dead space, transtracheal oxygen delivery can markedly reduce oxygen flow rates. Oxygen tanks last longer, allowing children to perform daily activities without having to change the oxygen tanks as often. Schooling may be less interrupted. Second, transtracheal oxygen delivery may have averted hospitalizations in some of our patients during episodes of higher oxygen demands, such as lower respiratory tract infections. Third, the discomfort, inconvenience, and social stigmata associated with nasal cannula use lead to decreased patient adherence. This is especially true in children. Finally, nasal cannulas frequently become dislodged during sleep, and are aesthetically less appealing.

Other advantages of TTOC use in children can be theorized. It is possible that the increased exercise tolerance and relief of dyspnea associated with transtracheal oxygen delivery in adults[14,15] is related to a decrease in respiratory work. In children, this decreased respiratory work may translate into an overall decrease in energy expenditure and hypothetically into improved growth patterns. The issue remains to be studied. A prospective case series may be warranted, especially in children with BPD, in whom increased work of breathing comes at the expense of growth.

The issue of how transtracheal oxygen delivery in children affects the cost of care remains to be answered. If a preexisting stoma is not present, insertion of the TTOC in children requires a costly trip to the operating room for bronchoscopy and placement. Children with TTOCs may require closer monitoring and more frequent outpatient visits. On the other hand, the improved efficiency of transtracheal oxygen delivery may even out the total cost of oxygen use in the long run.[8]

In conclusion, transtracheal oxygen delivery can be a reasonable alternative to a nasal cannula or formal tracheotomy in selected pediatric patients in whom long-term oxygen delivery, but not an alternate airway, is required. In order to avoid complications, meticulous technique must be adhered to in using the percutaneous approach for placement. The patients considered must have motivated caregivers who can be taught proper techniques to care for the TTOC and avoid possible complications. The improved oxygen delivery efficiency, aesthetic satisfaction, and usage comfort of the TTOC seem to promote patient adherence. These advantages, reported in adults, are also seen in children.

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MANAGEMENT OF THYROID NODULAR DISEASE AND CANCER

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ANALYSIS OF LOCAL RECURRENCE IN PATIENTS WITH SELECTED T1-3N0M0 SQUAMOUS CELL CARCINOMA OF THE TRUE VOCAL CORD MANAGED WITH A PLATINUM-BASED CHEMOTHERAPY–ALONE REGIMEN FOR CURE

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Based on an inception cohort of 35 patients with T1-3N0M0 squamous cell carcinoma of the true vocal cord who had a complete clinical response after a platinum-based induction chemotherapy regimen and a minimum of 3 years of follow-up, the current retrospective study documented the long-term results and consequences of local recurrence following the use of a platinum-based chemotherapy–alone regimen for cure. During the years 1985 to 1996, 231 patients with invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0 were managed at our department with a platinum-based induction chemotherapy regimen. A complete clinical response was achieved in 77 patients. Thirty-five of the 77 patients with complete clinical response were managed at our institution with a platinum-based chemotherapy–alone regimen. The statistical analysis of data on survival, local control, nodal control, distant metastasis, and metachronous second primary tumor incidence was based on the Kaplan-Meier product limit method. Univariate analysis was performed for potential statistical relation between local recurrence and various variables. The 3- and 5-year actuarial survival estimates were 91.4% and 88.6%, respectively. Overall, the causes of death were intercurrent disease in 6 patients and metachronous second primary tumor in 4 patients. The 3- and 5-year actuarial local control estimate was 64.8%. No significant statistical relation could be demonstrated between the incidence for local recurrence and the variables under analysis. Salvage treatment in patients with local recurrence yielded a 100% local control rate and laryngeal preservation rate. The 3- and 5-year actuarial lymph node control estimate was 97.1%. The 3- and 5-year actuarial estimate for patients with distant metastasis was 0%. The 5- and 10-year actuarial estimates for patients with metachronous second primary tumor were 9.7% and 28.1%, respectively. Although local recurrence was noted in almost a third of patients with complete clinical response who were managed with a platinum-based chemotherapy–alone regimen, it did not appear to be detrimental, as none of the patients who had local recurrence ultimately died from their disease or lost their larynx.

KEY WORDS — carcinoma, chemotherapy, true vocal cord.

INTRODUCTION

Partial laryngectomy and radiotherapy are the current conservative treatment methods advocated worldwide for patients with invasive squamous cell carcinoma of the true vocal cord. In the early 1980s, one of us (H.L.), at Laënnec Hospital, pioneered the concept and use of a platinum-based induction chemotherapy regimen in patients with invasive squamous cell carcinoma of the true vocal cord.[1,2] The previously reported experience gained with such an approach before the completion of partial laryngectomy suggested a strong statistical relationship between the achievement of a complete clinical response and a complete histologic regression of the tumor.[3-6] This notion progressively led us to offer a platinum-based chemotherapy–alone regimen for cure (also termed exclusive chemotherapy) in patients with invasive squamous cell carcinoma of the glottis when a complete clinical response was achieved after the induction chemotherapy regimen.[7]

In a prior report, we analyzed the results achieved with chemotherapy alone versus the conservative treatment methods of radiotherapy or surgery in patients with selected glottic carcinoma classified as T1-3 who had a complete clinical response after a platinum-based induction chemotherapy regimen.[7] The data reported noted that the use of a chemotherapy-alone regimen did not decrease the survival rate and allowed for a high rate of definitive cure.[7] However, local recurrence was noted in almost a third of cases.[7] This high rate of local recurrence was the initial impetus for the current study, which was designed to analyze the consequences and management of local recurrence and to document the long-term results in patients with selected T1-3N0M0 squamous cell carcinoma of the true vocal cord who had a complete clinical response and were managed with a platinum-based chemotherapy–alone regimen for cure.

PATIENTS AND METHODS

During the years 1985 to 1996, 231 patients with invasive squamous cell carcinoma of the true vocal

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TABLE 1. GENERAL DATA OF 35 PATIENTS

Age (y)	
Mean	62
Range	41-78
Age > 65 y (%)	28.5
Sex (%)	
Male	91.4
Female	8.6
Charlson et al[9] comorbidity index weighted score (%)	
0	57.2
1	20
2	11.4
3	5.7
4	5.7

TABLE 2. CUMULATIVE TOXICITY

	Grade				
Toxicity	*0*	*1*	*2*	*3*	*4*
Leukopenia	80	5.7	11.4		2.9
Thrombocytopenia	74.3	8.5	14.3		2.9
Anemia	68.6	11.4	17.1	2.9	
Stomatitis	74.3	8.6	17.1		
Nausea and/or vomiting	54.3	22.9	17.1	5.7	
Renal	37.2	31.4	20	11.4	
Cutaneous	97.1	2.9			
Neurologic	94.3		2.8	2.8	

Grading is according to Eastern Cooperation Oncology Group criteria.[10] Data are percentages.

cord classified as T1-3N0M0, according to the 1997 American Joint Committee on Cancer staging classification system,[8] were consecutively managed at our department with a platinum-based induction chemotherapy regimen. A complete clinical response, defined as the disappearance of the tumor with normal motion of the true vocal cord, negative biopsies at the time of laryngoscopy, and a normal imaging study of the larynx, was achieved in 77 patients (33.3%).

The current retrospective studies were based on analysis of the medical charts of 35 of the 77 patients with a complete clinical response who were managed at our institution with a platinum-based chemotherapy–alone regimen. The initial tumor was classified as T1N0M0 in 9 patients, T2N0M0 in 21 patients, and T3N0M0 in 5 patients. None of the patients within our series presented initially with tumor limited to the middle third of the mobile true vocal cord, with tumor originating from the anterior or posterior commissure, or with tumor considered as being transglottic, infraglottic, or supraglottic in origin. From a surgical point of view, the initial tumor was considered to be amenable to partial laryngectomy in 33 patients and to total laryngectomy in 2 patients. The gender, age, percentage of patients older than 65 years of age, and Charlson et al[9] comorbidity index–weighted score for the entire population are presented in Table 1.

The decision to undergo a platinum-based chemotherapy–alone regimen for cure was made after informed consent by patients who were fully aware of the modification from the standard conservative treatment options (partial laryngeal surgery or radiotherapy) conventionally advocated worldwide. Our current platinum-based chemotherapy–alone regimen for cure consisted of cisplatin (20 mg/m^2 per day) and fluorouracil (1 g/m^2 per day) delivered during a 6-day course with a 15- to 21-day interval between courses. The drug delivery was achieved via a long-term venous access device implanted within the subclavian vein at the time of initial panendoscopy. An infuser provided for a constant delivery of drugs during the courses. Antiemetics were used routinely during the courses. A regular intake of oral fluids (2 L/d) was achieved during the courses in order to prevent renal failure. The dosages of the drugs were adjusted to tolerance and toxicity. The toxicity determined the interval between courses. Because of renal toxicity, the cisplatin was changed to paraplatin in 9 patients. The cumulative toxicity according to the Eastern Cooperative Oncology Group[10] criteria is presented in Table 2. The audiological toxicity was not systematically evaluated. The experience gained at our institution, together with the toxicity noted, led to variations in the overall number of courses performed. Nine patients (most of them in the early years) underwent 7 to 11 courses. Twenty-three patients underwent 5 to 6 courses; this is the number of courses that we advocate at the present time for patients with a complete clinical response who are managed with platinum-based chemotherapy alone. As a result of the toxicity, 3 patients underwent only 4 courses. Because of the variations in the total number of courses performed, the total dosage of cisplatin delivered varied from 140 to 1,320 mg (mean dosage, 740 mg), the total dosage of paraplatin delivered varied from 280 to 3,600 mg (mean dosage, 360 mg), and the total dosage of fluorouracil delivered varied from 19 to 145 g (mean dosage, 45 g).

Follow-up data were collected during periodic visits to our clinic. Clinical examination was performed monthly during the first 2 years after treatment. Direct laryngoscopy was performed in patients with dysphonia lasting for more than 2 weeks or if laryngeal abnormalities were noted at the time of clinical examination. Chest radiography was performed yearly. All patients were followed for a minimum of 3 years or until death. No patients were lost to follow-up. Twenty-nine patients (82.9%) had a minimum of 5 years of follow-up. The statistical analysis of data on survival, local control, lymph node control, distant metastasis, and metachronous second primary tumor in-

TABLE 3. OUTCOME OF 12 PATIENTS WITH LOCAL FAILURE

Age (y)	*Sex*	*Initial T Stage*	*Nodal Recurrence*	*Metachronous Second Primary*	*Outcome*
78	M	T2	13 mo		Died at 7 y of intercurrent disease
73	M	T2			Alive at 14 y without evidence of disease
55	M	T2		46 mo (liver)	Died at 5 y of metachronous second primary
44	M	T1			Alive at 9 y without evidence of disease
62	M	T3			Alive at 8 y without evidence of disease
56	M	T1		74 mo (glottis)	Alive at 9 y without evidence of disease
53	M	T2			Alive at 8 y without evidence of disease
77	M	T2		34 mo (trachea)	Died at 3 y of metachronous second primary
60	M	T2	36 mo		Alive at 5 y without evidence of disease
50	M	T2			Alive at 3 y without evidence of disease
41	F	T2			Alive at 3 y without evidence of disease
77	M	T2			Alive at 3 y without evidence of disease

cidence was based on the product-limit method of Kaplan and Meier.[11] We performed univariate analysis of the correlation of local recurrence with the following factors: age, gender, comorbidity index, T stage, true vocal cord motion, total dosage of cisplatin, total dosage of paraplatin, and total dosage of 5-fluorouracil. The parametric χ^2 test and the nonparametric Mann-Whitney U test and Kruskal-Wallis H test were used for analysis of qualitative and quantitative variables, respectively. Significance was determined by a p value of less than .05.

RESULTS

The 3- and 5-year actuarial survival estimates were 91.4% and 88.6%, respectively. Overall, 10 patients died in our series. The cause of death was intercurrent disease in 6 patients and metachronous second primary tumor in 4 patients. Toxicity related to the platinum-based chemotherapy–alone regimen (Table 2) and/or local recurrence never resulted in death.

The 3- and 5-year actuarial local control estimate was 64.8%.The overall crude incidence of local recurrence was 34.3% (12 of 35). No significant statistical relationship could be demonstrated between local control and the variables under analysis (age, gender, comorbidity index, T stage, true vocal cord motion, total dosage of cisplatin, total dosage of paraplatin, and total dosage of 5-fluorouracil). Local recurrence was always noted within the first 18 months after the end of the platinum-based chemotherapy–alone regimen. Pathological analysis revealed that the recurrent local tumor was in situ carcinoma in 2 patients, microinvasive squamous cell carcinoma in 3 patients, and *early* stage (T1-2) invasive squamous cell carcinoma in 7 patients. The management of local recurrence was partial laryngectomy in 10 patients (laser excision in 2 and an open surgical approach in 8 patients) and radiotherapy in 2 patients. Table 3 presents the outcome of the 12 patients who had local failure. The successful management of the 12 patients who had local failure after the platinum-based chemotherapy–alone regimen resulted in an overall local control and laryngeal preservation rate of 100% (Table 3).

The 3- and 5-year actuarial nodal control estimate was 97.1%. The 3- and 5-year actuarial estimate for distant metastasis was 0%. The overall crude incidence for nodal recurrence was 5.7% (2 of 35). Nodal recurrence was noted only in patients who had local recurrence (Table 3). The nodal recurrence was jugulocarotid and ipsilateral to the initial tumor. Successful management with surgery and postoperative radiotherapy in the 2 patients in whom jugulocarotid lymph node recurrence developed resulted in an overall 100% nodal control rate. The 5- and 10-year actuarial estimates for the development of a metachronous second primary tumor were 9.7% and 28.1%, respectively. The overall crude incidence of the development of a metachronous second primary tumor was 20% (7 of 35). The metachronous second primary tumor involved the supraglottic larynx in 1 patient, the glottic larynx in 1, the oropharynx in 1, the trachea in 1, the lung in 2, and the liver in 1. In the patient who had a metachronous second primary of the glottic larynx, the lesion developed at the exact same location of the initial carcinoma, more than 6 years after the end of the platinum-based chemotherapy–alone regimen. Successful management of the metachronous second primary tumor was achieved in 3 of the 7 patients.

DISCUSSION

Five concepts support the notion that a platinum-based chemotherapy–alone regimen might be a valuable conservative treatment option in patients with invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0 who have a complete clinical response after induction chemotherapy. 1) There is a need for new treatment approaches in patients with invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0. 2) Induction chemotherapy yields a low rate of toxicity. 3) Induction chemotherapy results in a high rate of complete clinical response. 4) Chemotherapy alone for cure in patients with a complete clinical response achieves a high rate of local control. 5) Local recurrence after chemotherapy alone is not detrimental to survival and remains amenable to successful salvage treatment. In the current report we will discuss each of these 5 concepts and develop the notion that there is a possible innovative and interesting role for a platinum-based induction and chemotherapy–alone regimen in patients with selected invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0.

In the past 20 years, various groups over the world have been proponents of the use of platinum-based induction chemotherapy regimens in patients with *advanced* laryngeal carcinoma. Certainly, the multi-institutional prospective randomized trial by the US Department of Veterans Affairs Laryngeal Cancer Study Group was a major breakthrough for the use of induction chemotherapy regimens in patients with *advanced* laryngeal cancer.[12] Although no survival benefit could be observed in a platinum-based induction chemotherapy regimen in patients with *advanced* laryngeal cancer, the Department of Veterans Affairs study demonstrated that in patients with a clinical reponse to the induction chemotherapy regimen, total laryngectomy could be avoided and radiotherapy used to achieve tumor control and laryngeal preservation without a detrimental effect in terms of survival.[12] On the other hand, there is a general belief, recently summarized by Wolf and Hong,[13] that there is no role for a platinum-based induction chemotherapy regimen in patients with *early* laryngeal cancer that is amenable to radiotherapy or partial laryngectomy. We certainly agree that the results achieved with partial laryngectomy or radiotherapy alone are highly efficient in patients with laryngeal carcinoma limited to the middle third of the mobile true vocal cord classified as T1. However, when the tumor of the true vocal cord reaches the anterior commissure, involves regions adjacent to the glottis, and/or causes impaired motion or fixation, the results achieved with radiotherapy or partial laryngectomy alone are far from satisfactory in terms of local control. The local recurrence rate in patients with glottic tumors classified as T1 with anterior commissure invasion has been reported to vary from 14% to 28% after vertical partial laryngectomy or radiotherapy.[4] In large series with a long follow-up, the local recurrence rate has been reported to vary from 22% to 43% in patients with tumors classified as T2 and from 9% to 64% in patients with tumors classified as T3 (because of fixation of the true vocal cord) after vertical partial laryngectomy or radiotherapy alone.[3-6] Furthermore, studies based on large series with a long duration of follow-up that were specifically designed to document the consequences of local recurrence after the use of radiotherapy or partial laryngectomy in patients with invasive squamous cell carcinoma of the glottis have demonstated that local recurrence was highly detrimental in terms of laryngeal preservation and long-term survival.[14-16] In our opinion, such data support the notion that in most patients with *early* invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0, the results achieved with the single treatment method of vertical partial laryngectomy or radiotherapy alone are far from optimal in terms of local control, laryngeal preservation, and survival, and support the notion that there is clearly a need for new approaches. The use of a platinum-based induction chemotherapy regimen was viewed at our institution as an option, among others such as hyperfractionation techniques, concomitant chemotherapy, and extensive partial laryngeal surgery (ie, near-total laryngectomy with epiglottic pull-down or supracricoid partial laryngectomy), that could allow for an improvement in overall local control. Such a concept appeared to be valuable, as data from our department over the past 15 years constantly noted that the use of platinum-based induction chemotherapy before partial laryngeal surgery caused an increase in the local control rates of patients with invasive squamous cell carcinoma of the glottis not limited to the middle third of the mobile true vocal cord and classified as T1-3N0M0.[1-6]

The second concept supporting a possible role for a platinum-based chemotherapy–alone regimen in patients with invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0 was the low toxicity encountered in such a treatment option. Again, there is a general belief in laryngology that the use of a platinum-based induction chemotherapy regimen in patients with *early* stage laryngeal cancer results in unnecessary morbidity and mortality. Such a notion is supported by the meta-analysis performed in the late 1980s by Stell and Rawson,[17] who noted that the mortality rate associated with adjuvant chemotherapy regimens in head and neck cancers averaged 6.5%. However, the trials analyzed in this meta-analysis were extremely heterogeneous in terms of drugs and regimens used, populations analyzed, tumor stages, medical histories, and the patients' status and goals.[17] Furthermore, the meta-analysis type of study, although indicative, is far from being recognized in medicine as the gold standard from a scientific and statistical point of view. The clinical experience at our institution in terms of toxicity encountered in head and neck carcinoma with a platinum-based induction chemotherapy regimen does not parallel the data reported by Stell and Rawson,[17] as the mortality rate associated with induction chemotherapy regimens was noted to be 0.5% in a series of 1,100 patients with head and neck squamous cell carcinoma.[18] More recently, the use of induction chemotherapy did not result in a single death in a series of 100 patients with invasive squamous cell carcinoma of the true vocal cord classified as T2.[5] Similarly, in the current study, severe toxicity was uncommon (Table 2), and the use of chemotherapy never resulted in death. This apparent low toxicity achieved at our institution with a platinum-based chemotherapy regimen might be explained by various factors: the combination of only 2 drugs (platinum and fluorouracil), the use of a portable chemotherapy delivery system that provides a constant flow of drugs over a 24-hour period, the systematic prevention of renal and digestive toxicities with the completion of oral hyperhydration and the use of antiemetic drugs during the courses, the drug dosage adjustments to clinical and biological tolerance, and the cessation of chemotherapy (induction or exclusive) in patients who presented with grade 3 or higher toxicity according to the Eastern Cooperative Oncology Group criteria.[10] However, as depicted in Table 2, grade 3 and 4 toxicity was noted in a few patients; therefore, even though chemotherapy was well tolerated overall in this inception cohort, one must not expect that the mortality rate from chemotherapy would remain zero if a larger number of patients was included.

The third concept to advocate a possible role for a platinum-based chemotherapy–alone regimen for cure in patients with invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0 was the achievement of a high rate of complete clinical response after the completion of induction chemotherapy. As noted in the Materials and Methods section, the estimate of the complete response rate of the patients with squamous cell carcinoma of the true vocal cord classified as T1-3N0M0 managed at our department during the years 1985 to 1996 with a platinum-based induction chemotherapy regimen was 33.3% (77 of 231). This figure is in line with the 30% estimate reported at the onset of our experience.[7] Such a percentage is similar to the 31% rate of complete clinical response noted in the Department of Veterans Affairs study[19] devoted to *advanced* stage laryngeal cancer. This finding conflicts with the idea that patients with smaller tumors have a higher response rate, as suggested in the early 1980s by Wolf et al,[20] who noted, in a prospective randomized multicenter study of 283 patients with resectable head and neck cancers, that a complete clinical response was more commonly noted in patients with tumors classified as T1-2 than in patients with tumors classified as T3-4.

In this study, the overall crude incidence of local recurrence was 34.3% (12 of 35), and the 3- and 5-year actuarial local control estimate after exclusive chemotherapy alone was 64.8%. In comparing this last figure with the 33.3% rate (77 of 231) of complete clinical response achieved at our department with platinum-based induction chemotherapy, it appears that chemotherapy regimen could cure 19.5% of patients with invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0. Such figures are in line with the data previously reported at the onset of our experience with a platinum-based chemotherapy–alone regimen for patients with glottic carcinoma who have a complete clinical response.[7] Furthermore, the fact that about two thirds of patients (23 of 35) with invasive squamous cell carcinoma of the true vocal cord who had a complete clinical response after our platinum-based induction chemotherapy regimen were definitively cured with exclusive chemotherapy validates, in our opinion, the concept that exclusive chemotherapy can be of use in these patients. Interestingly, in our series, none of the variables under analysis (Table 2) appeared to be statistically related to local control data. Such data suggested, in our opinion, that the efficacy of platinum-based chemotherapy was not related to the total dosage of drugs delivered, but rather, relied on not-yet-elucidated mechanisms. This notion has led us to progressively reduce the number of courses given in an exclusive chemotherapy regimen; the total number of courses now advocated by our department is 5 or 6.

The last concept to advocate the use of exclusive chemotherapy alone in patients with squamous cell carcinoma of the true vocal cord classified as T1-3N0M0 who have a complete clinical response is the fact that local recurrence should not be detrimental to survival and should remain amenable to successful salvage treatment. At the onset of our experience with a platinum-based chemotherapy–alone regimen, we demonstrated that the use of radiotherapy or partial laryngeal surgery in glottic carcinoma patients with a complete clinical response after a platinum-based induction chemotherapy regimen resulted in a statistically higher local control rate.[7] Moreover, the 12 patients who had local recurrence in the current series were successfully managed with a conservative conventional laryngeal treatment option (laser excision in 2, conventional partial laryngectomy in 8, and radiotherapy in 2), resulting in an overall 100% local control and laryngeal preservation rate (Table 3). In our opinion, such a high-rate of successful salvage by conservative laryngeal treatment is related to early detection of local recurrence and the oncological behavior of glottic carcinoma. In our series, local recurrence was always detected within 18 months of the end of the exclusive chemotherapy regimen. This early detection of local recurrence was enhanced by a monthly follow-up clinical examination performed during the first 2 years after treatment, together with direct laryngoscopy performed in patients with dysphonia lasting for more than 2 weeks and/or in whom laryngeal abnormalities were noted at the time of clinical examination. The pathology data confirmed the early detection of the recurrent local tumors: in situ carcinoma in 2 patients, microinvasive squamous cell carcinoma in 3 patients, and *early* stage (T1-2) invasive squamous cell carcinoma in 7 patients. It is also very interesting to note that local recurrence after exclusive chemotherapy was not detrimental in terms of nodal recurrence, distant metastasis, or survival rate as compared to local recurrence after radiotherapy or partial laryngectomy. The 3- and 5-year actuarial estimate for distant metastasis was 0%, and the 3- and 5-year actuarial nodal control estimate was 97.1% in our series. Furthermore, none of the patients in our series died of their initial tumor; intercurrent disease (6 patients) and metachronous second primary tumors (4 patients) were the causes of death. Such figures are far better than the nodal recurrence and distant metastasis rates reported by Viani et al[15] in patients with glottic carcinoma in whom radiotherapy failed and by Laccourreye et al[16] in patients with glottic carcinoma in whom vertical partial laryngectomy failed. These differences suggest that when invasive squamous cell carcinoma of the true vocal cord recurred locally after a chemotherapy-alone regimen, the recurrent tumor behaved as the initial tumor, with a low risk of nodal recurrence or distant metastasis. Such data also explain the good survival rates noted in our series: the actuarial survival estimates were 91.4% at 3 years and 88.6% at 5 years.

In conclusion, the data from our current series confirm the data previously reported from our institution[7] suggesting that our current platinum-based chemotherapy–alone regimen cures a non-negligible group of patients with invasive squamous cell carcinoma of the true vocal cord classified as T1-3N0M0 who have a complete clinical response after an induction chemotherapy regimen. In our opinion, this result should be viewed as a major breakthrough in head and neck oncology, as it nullifies the old concept that head and neck squamous cell carcinomas cannot be cured with chemotherapy. We are well aware that the retrospective nature of our report prevents any definitive conclusion and that some might argue that a 15% to 20% estimate for definitive cure is not worth the initial morbidity, toxicity, and cost related to the use of chemotherapy regimens as compared to the conventional conservative treatment methods of radiotherapy or partial laryngeal surgery alone. To us, the concept of curing 15% to 20% of patients with invasive squamous cell carcinoma of the glottis while preserving radiotherapy and/or surgery for the management of local recurrence or a metachronous second primary tumor is valuable enough to discuss with the patient the use of an induction chemotherapy regimen. Future studies should be aimed at 1) determining whether this new concept of curability with chemotherapy might be extended to other laryngeal subsites or other head and neck sites, 2) evaluating whether the introduction of other drugs could improve the clinical response rate and overall curability, and 3) studying the mechanism of drug resistance in an attempt to define and select the tumors most amenable to induction chemotherapy.

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EDITORIAL COMMENT

The serendipitous observation was made by Dr Laccourreye and his group that many patients treated for squamous cell carcinoma of the vocal cord with induction chemotherapy who went on to partial laryngeal surgery ended up not having any tumor in the surgical specimen. This observation led to a platinum-based chemotherapy-alone regimen, intended for cure, in patients with invasive squamous cell carcinoma (T1-3). In a later report, the authors analyzed the results achieved with chemotherapy alone as compared with radiotherapy or conservation surgery in patients with selected glottic carcinoma (T1-3); they noted that many (5) patients had a complete clinical response after a platinum-based induction chemotherapy regimen. They found that the use of a chemotherapy-alone regimen did not decrease the survival rate and not only allowed for a high rate of cure, but also resulted in organ preservation and good function. The authors did note that there was local recurrence in almost one third of the patients who were treated for cure with chemotherapy.

This current publication reports a study that was designed to analyze the management of local recurrence and to document the long-term results in patients with T1-3N0M0 squamous cell carcinoma of the true vocal cord who were managed with a platinum-based chemotherapy-alone regimen for cure and had a complete clinical response. Early in their series, they submitted many of these patients to 7 to 11 courses of chemotherapy, but now they have been able to reduce them to 5 or 6 courses of chemotherapy, which is their current regimen. Overall, the 3- and 5-year actuarial survivals reported were 91.4% and 88.6%, respectively. The cause of death in 10 patients in their series was intercurrent disease in 6 and metachronous second primaries in 4. Toxicity related to the chemotherapy and/or local recurrence never resulted in death. Local recurrence was always noted within the first 18 months after the chemotherapy-alone regimen. The management of local recurrence was partial laryngectomy in 10 patients and radiotherapy in 2 patients. It is interesting that the increase in local control rate in carcinoma of the glottis was in patients in whom the tumor was not limited to the middle third of the true vocal cord. The authors state that while a few patients experienced grade 3 or 4 toxicity, chemotherapy overall was well tolerated. No one died directly as a result of this treatment. Twenty-three of 35 patients with cancer of the true vocal cord who had a complete clinical response after induction chemotherapy were cured exclusively with chemotherapy when the patients were followed long after their treatment program.

The 12 patients who had local recurrence in this series were successfully managed with conservation treatment resulting in an overall 100% local control laryngeal preservation rate. In the authors' opinion, such a high rate of successful salvage by conservation treatment is related to early detection of local recurrence. They concluded that early detection of local recurrence was enhanced by a monthly follow-up clinical evaluation performed by an otolaryngologist during the first 2 years after treatment, together with direct laryngoscopy performed in patients with dysphonia lasting for more than 2 weeks or if clinical abnormalities were noted at the time of clinical examination.

This is an intriguing approach and potentially a very major paradigm shift in the treatment of early-stage cancers of the glottis without metastasis to the neck. Since regional recurrence in such patients is usually not the major issue, the ability to cure patients with this cancer with preservation of the voice is a major achievement. While the study is retrospective, the results are from a group that has, for many years, dealt with a high volume of patients with carcinoma of the larynx and whose publications in the area of voice-sparing surgical techniques have been well accepted.

The major advantages of this approach are a high degree of curability, functional organ preservation, and the possibility of keeping radiotherapy in reserve for metachronous primaries such as those reported in this series. It would be of major importance to have these results validated in prospective analyses by these authors and others. There is at least one institution in the United States that is studying this approach of chemotherapy alone for cure, and in a small series, it has validated the above-mentioned results.

One of the key features of the authors' success in the treatment of recurrence is that because of the frequent follow-up by the otolaryngologist, the cancer is diagnosed early. I have concern that in the community practice, once these patients are referred to a medical oncologist, their access to the otolaryngologist for careful follow-up and immediate evaluation of suspected recurrence and treatment of recurrent disease would be delayed, and therefore the results described in this excellent series would be downgraded.

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ISOLATED SPHENOID SINUS DISEASE: AN ANALYSIS OF 122 CASES

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Isolated sphenoid sinus disease (ISSD) is a relatively uncommon disease. The present study is a retrospective review of 122 patients with ISSD who were treated at the Department of Otolaryngology, Eye, Ear, Nose and Throat Hospital at Shanghai Medical University over a 25-year period. The diagnosis of ISSD was made on the basis of history and physical examination, signs and symptoms, nasal endoscopy, and computed tomography (CT) and magnetic resonance imaging (MRI). The final diagnosis of ISSD was confirmed by histopathologic and microbiological examinations of the surgical specimens. The pathological findings in this study included sphenoid cyst (47 cases), sphenoid sinusitis (31 cases), fungal disease (19 cases), inverted papilloma (4 cases), sphenochoanal polyp (1 case), foreign body (8 cases), malignant tumors (8 cases), and others (4 cases). The most common initial symptom was headache, followed in decreasing order by visual changes, cranial nerve palsies, and nasal symptoms. The more frequent use of routine CT and MRI scanning, as well as endoscopy, in the diagnosis of sinus disease has led to an increase in the early diagnosis of ISSD. The recent advances in endoscopic sphenoidotomy has allowed for relatively safe and immediate treatment of ISSD, preventing late extension into adjacent vital structures, which is commonly fatal. Endoscopic surgery also enables the surgeon to make a precise pathological diagnosis.

KEY WORDS — computed tomography, headache, magnetic resonance imaging, nasal endoscopy, sphenoid sinus, sphenoidotomy.

INTRODUCTION

Isolated sphenoid sinus disease (ISSD) is an uncommon disease that affects 1% to 2.7% of patients with diagnoses of paranasal sinus disease.[1-10] In 1973, Wyllie et al[1] reviewed 45 patients with ISSD treated at the Mayo Clinic over a 37-year period and defined ISSD as sinus disease isolated solely to the sphenoid sinus. Since then, only a few reports concerning ISSD have been published (Pearlman et al,[2] 43 cases; Rothfield et al,[3] 13 cases; Hnatuk et al,[4] 3 cases). Interestingly, the number of patients with ISSD in more recent reports has been increasing (Gilain et al,[5] 14 cases; Hadar et al,[6] 21 cases; Lawson and Reino,[7] 132 cases; Sethi,[8] 21 cases).

The onset of ISSD is often insidious. The history and physical examination contribute little to establishing the correct diagnosis. Plain radiologic examination of the sphenoid sinus is also inadequate to make a diagnosis of ISSD, even when additional tomography is used. Therefore, before computed tomography (CT) was available, a missed or delayed diagnosis of ISSD often resulted in serious complications or death. With the recent advent of CT, magnetic resonance imaging (MRI),[11,12] nasal endoscopy,[13-16] and functional endoscopic sinus surgery,[14-17] a great deal of progress has been made in the diagnosis, treatment, and prognosis of this rare disease. In the present study, 122 patients with ISSD were evaluated with regard to their presenting signs and symptoms, appearances of radiographic images, and surgical treatments in order to investigate the role of endoscopic ostial sphenoidotomy in improving the diagnosis, treatment, and prognosis of ISSD.

MATERIALS AND METHODS

The hospital charts of 122 patients with a diagnosis of ISSD were retrospectively reviewed. All 122 patients were treated at the Department of Otolaryngology, Eye, Ear, Nose and Throat Hospital, Shanghai Medical University, over a period of 25 years (1973 to 1998). The cohort consisted of 69 male patients and 53 female patients with ages ranging from 4 to 77 years (mean, 44.7 years). The diagnosis of ISSD was based on characteristic signs and symptoms, radiographic imaging, and histopathologic and microbiological examinations of the resected specimens. All patients underwent radiographic examination: 21 patients were evaluated by plain radiography including tomography, 92 patients underwent CT, and 22 patients underwent MRI. All radiographic

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TABLE 1. PATHOLOGICAL FINDINGS IN ISSD BY TIME PERIOD

Disease	*1973-1978*	*1979-1983*	*1984-1988*	*1989-1993*	*1994-1998*	*Total*
Cyst	2	5	7	13	20	47
Sinusitis	1	2	2	7	19	31
Fungal infection	0	0	3	5	11	19
Foreign body	2	1	3	1	1	8
Carcinoma	1	2	1	2	1	7
Inverted papilloma	1	0	0	1	2	4
Fibro-osteoma	1	1	0	0	1	3
Polyp	0	0	1	0	0	1
Hemangioma	1	0	0	0	0	1
Lymphoma	0	0	0	0	1	1
Total	9	11	17	29	56	122

Data are numbers of patients.

ISSD — isolated sphenoid sinus disease.

TABLE 2. SYMPTOMS AND SIGNS OF ISSD

Disease (n)	*Headache*	*Visual Changes*	*Eye Movement*	*Epistaxis*
Cyst (47)	30	18	20	3
Sinusitis (31)	20	6	1	11
Fungal infection (19)	12	1	0	15
Foreign body (8)	2	2	0	1
Carcinoma (7)	5	3	0	4
Inverted papilloma (4)	2	0	1	2
Fibro-osteoma (3)	2	3	3	0
Polyp (1)	1	0	0	0
Hemangioma (1)	1	0	0	1
Lymphoma (1)	1	1	1	1
Total (122)	76 (62%)	34 (28%)	26 (21%)	38 (31%)

studies revealed a space-occupying lesion in the sphenoid sinus, including the cells of Onodi. The radiographs were also evaluated for intrasinus opacification, sinus expansion and thinning, remodeling, and erosion of the bony wall with extrasinus spread to nearby structures. The follow-up period was at least 12 months in all patients.

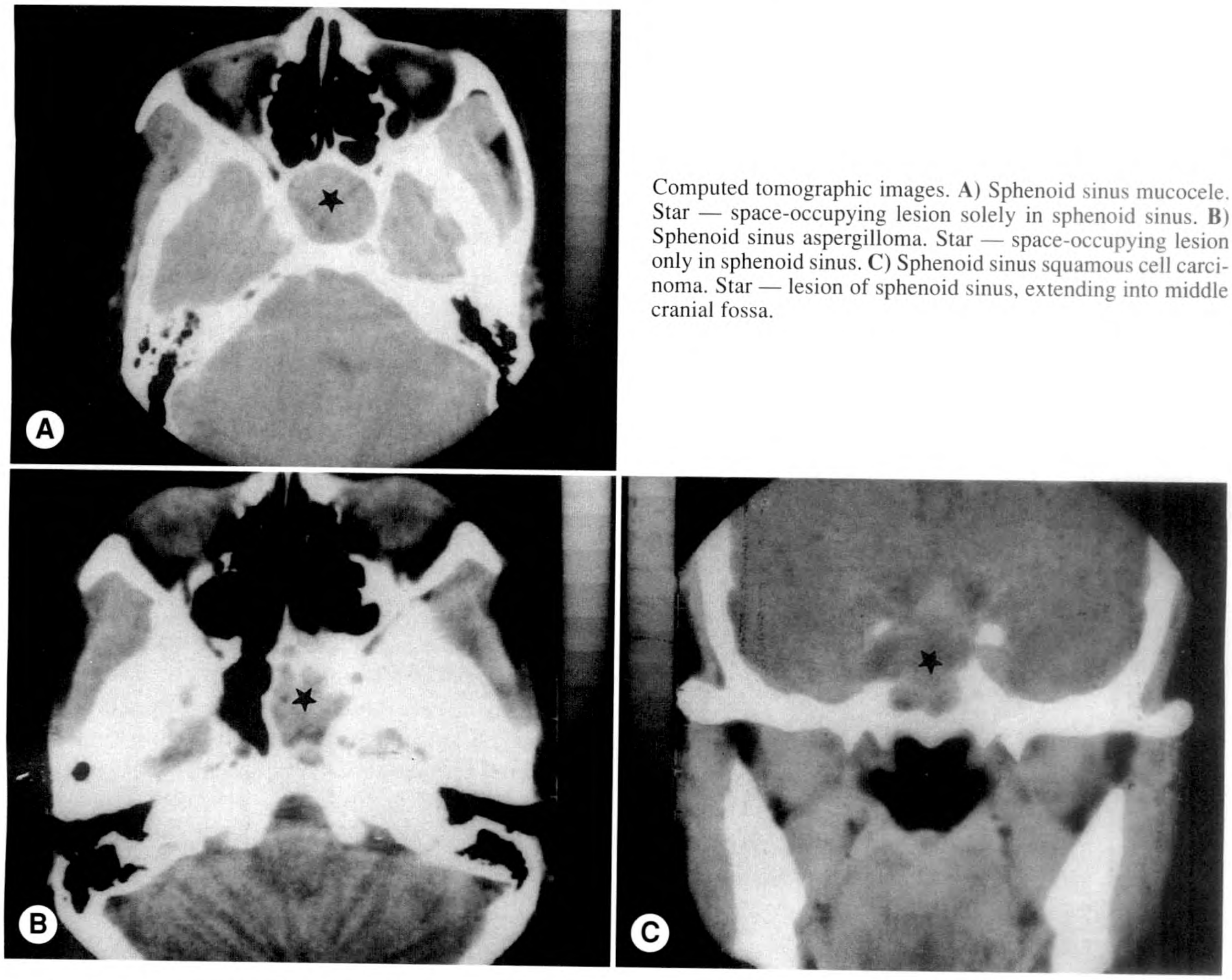
RESULTS

Pathology. Histopathologic analysis of the surgical specimens revealed 98 cases of inflammatory disease, 8 cases of benign tumors, 8 cases of foreign bodies, and 8 cases of malignant tumors. Of the 98 cases in the inflammatory disease group, 47 cases were diagnosed as cysts, 31 cases were diagnosed as sinusitis, 19 cases were diagnosed as fungal sinusitis, and 1 case was diagnosed as polyp disease. Of the 8 cases in the benign tumor group, 4 cases were diagnosed as papilloma, 3 cases were diagnosed as fibro-osteoma, and 1 case was diagnosed as hemangioma of the sphenoid sinus. Of the 8 cases in the malignant tumor group, 7 cases were diagnosed as carcinoma, and 1 case was diagnosed as malignant lymphoma (Table 1). In the series of 122 patients diagnosed and treated over a period of 25 years, 9 were treated in the years 1973 to 1978, 11 in the years 1979 to 1983, 17 in the years 1984 to 1988, 29 in the years 1989 to 1993, and 56 in the years 1994 to 1998. After the introduction of CT and MRI in 1984 and nasal endoscopy in 1986, the number of patients with the diagnosis of ISSD greatly increased, with increased numbers of cysts, sinusitis, and fungal sinusitis. However, the morbidity of neoplasms of the sphenoid sinus remained the same.

Symptoms and Signs. The most common symptom of ISSD was headache refractory to medical management (Table 2). Seventy-six patients (62%) complained of intractable headache. The predominant site of the headache was nonspecific, but it usually involved the frontal, temporal, retro-orbital, or retrobulbar regions. In several cases, the headaches were migratory and multifocal. Ocular symptoms were the second most common complaint. Thirty-four patients (28%) had visual disturbances, including visual loss or blindness. Twenty-six patients (21%) suffered from palsies of cranial nerves III, IV, or V. Nasal symptoms were the third most common complaint; 38 patients (31%) presented with epistaxis. Epistaxis was especially common in patients with fungal sinusitis (15 of 19 or 79%).

Nasal Findings. The nasal cavity was examined before operation in all patients. Only 18 patients had positive nasal findings in the middle meatus, nasopharynx, or olfactory sulcus. However, with nasal endoscopy, several additional abnormal findings were identified in 79 patients, including swelling of the anterior wall of the sphenoid sinus or nasopharynx (70 cases), congestion or edema in the middle meatus (30 cases), and purulent discharge in the posterior middle meatus, olfactory sulcus, or nasopharynx (20 cases). Neoplasms at the ostium of the sphenoid sinus were detected in 2 patients.

Imaging. In all 122 cases, radiography showed a space-occupying lesion detected solely in the sphenoid sinus. In patients with a sphenoid sinus cyst, a low-density soft tissue mass without enhancement was seen in the sphenoid sinus, associated with obvious bone absorption (see Figure, A). Patients with sphenoid sinusitis presented with an air-fluid level and granuloma formation associated with bone sclerosis. Fungal sinusitis showed evidence of calcification within the sinus and sinus wall sclerosis (see Figure, B). Six patients with malignant disease within the sphenoid sinus (75%) were found to have a soft tissue mass in the sinus associated with adjacent bone



Computed tomographic images. **A**) Sphenoid sinus mucocele. Star — space-occupying lesion solely in sphenoid sinus. **B**) Sphenoid sinus aspergilloma. Star — space-occupying lesion only in sphenoid sinus. **C**) Sphenoid sinus squamous cell carcinoma. Star — lesion of sphenoid sinus, extending into middle cranial fossa.

erosion (see Figure, C). The 12 patients with benign disease of the sphenoid sinus (10%) demonstrated much less bone erosion than did patients with malignant disease. Magnetic resonance imaging was useful in determining the presence and extent of skull base invasion and intracranial involvement.

Treatment and Prognosis. Nasal endoscopic surgery (direct ostial sphenoidotomy) was performed on 79 patients. The lesion was safely removed and at the same time investigated by histopathology and microbiology. Drainage of the sphenoid sinus was established in all 79 patients, but in 4 patients, it subsequently became blocked after operation. Twenty-five patients underwent sphenoid surgery via an external approach. Four patients underwent intranasal sphenoid surgery, but 1 patient had recurrent disease after the operation. One patient underwent sphenoid sinus surgery through an anterior skull base approach. One patient was treated successfully with conservative therapy using antibiotics. Six patients with malignant tumors of the sphenoid sinus received combined therapy with irradiation and chemotherapy drugs. No complications occurred in the patients who underwent surgery. After operation, their headaches disappeared in 70 of 76 patients (92%), and visual disturbances improved in 15 of 34 patients (44%).

DISCUSSION

The sphenoid sinus is located deep in the skull base, within the sphenoid bone. The initial onset of ISSD is generally asymptomatic, and during the pre-CT era, the sphenoid sinus was called the most neglected paranasal sinus.[18,19] Proetz[20] pointed out the proximity of the sphenoid sinus to 13 vital and vulnerable structures: superiorly, the pituitary gland, the middle cranial fossa, and the optic nerve and chiasm; on both sides, the cavernous sinuses, the internal carotid arteries, and cranial nerves III, IV, V, and VI; and anteriorly, the nasopharynx, the pterygoid canals and nerves, and the pterygopalatine ganglion and artery. He emphasized that the symptoms of ISSD are often referred to these adjacent structures, rather than to the sinus itself. The most common symptom of ISSD was headache, with an incidence between 33% and 81%. In our series of 122 patients, as indicated in Table 2, the incidence was 62%. When stratified by disease, headache was found in 64% of patients with a sphenoid cyst, 65% of patients with sinusitis, 63% of patients with fungal sinusitis, 75% of patients with malignant tumors, and 56% of patients with benign tumors. Twenty patients presented with headache as the only symptom. Fourteen patients were first referred to the neurosurgeon for severe headache, but after radiographic imaging, ISSD was eventually diagnosed. The studies of Gilain et al[5] and Lawson and Reino[7] confirm our finding that headache was the most common symptom in ISSD. Headache was found in malignant and benign diseases in 90% and 71% of their patients, respectively. Sethi[8] reported that retro-orbital headaches were typical of ISSD. In the present study, however, the predominant site of headache was found to be nonspecific.

Patients with optic nerve involvement frequently present with visual loss and even blindness. The incidence of visual disturbance has been reported to range from 24% to 50%. In this study, visual loss was detected in 38% and 50% of the patients with benign and malignant tumors, respectively. Ten patients with visual loss were first referred to an ophthalmologist. Five patients were initially found to have optic nerve inflammation, but after further examination, ISSD was subsequently diagnosed. This delay in diagnosis is due to the intimate relationship between the optic nerve and the sphenoid sinus. The optic nerve frequently bulges into the sinus, and its bony wall may be thin or even dehiscent. However, once ISSD produces visual loss, emergency surgery should be performed to decompress the optic nerve and avoid irreversible nerve degeneration.

The nasal cavity is seldom involved in patients with ISSD. Lawson and Reino[7] demonstrated that only 4% of patients with ISSD presented with nasal bleeding, whereas patients with malignant neoplasms presented frequently with epistaxis (33%). In the present study, 38 of 122 patients had epistaxis. Fifteen of 19 patients (79%) with fungal sinusitis had nasal bleeding, whereas 5 of 8 patients (57%) with malignant neoplasms had epistaxis. Therefore, ISSD patients with epistaxis can generally be considered to have either a malignant tumor or fungal sinusitis.

The diagnosis of ISSD is based on radiographic imaging that locates a space-occupying lesion solely in the sphenoid sinus. Then, CT or MRI provides detailed information about ISSD, including anatomic considerations, extension of the disease, and destruction around the sphenoid sinus. As well, MRI can distinguish between inflammation and benign or malignant neoplasms, as well as identify intracranial involvement. Nasal endoscopy can also aid in the diagnosis and treatment of ISSD. In comparison to routine unaided anterior and posterior rhinoscopy, nasal endoscopy can identify subtle findings in the middle meatus, the olfactory sulcus, and the orifice of the sphenoid sinus. When ISSD was diagnosed in an early stage and anatomic landmarks were clearly identified, endoscopic ostial sphenoidotomy involved minimal blood loss, less pain, quick recovery from surgery, and a shorter operation time. With the increasing use of routine CT, MRI, and nasal endoscopy, ISSD can be diagnosed at an early stage and treated relatively safely without involvement of nearby structures that might have the potential for serious complications and death. Therefore, when we encounter patients with ISSD or patients with intractable headaches, we routinely perform endoscopic ostial sphenoidotomy in order to make an accurate diagnosis and remove the lesion safely. In the coming century, nasal endoscopic ostial sphenoidotomy will be a routine technique for the management of ISSD.

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EFFECT OF CHRONIC ELECTRICAL STIMULATION OF LARYNGEAL MUSCLE ON VOICE

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Conventional surgical therapies for bilateral laryngeal paralysis sacrifice voice to enlarge the airway. Electrical pacing of the posterior cricoarytenoid (PCA) muscle to restore glottal opening and allow ventilation offers a new treatment approach. The purpose of this investigation was to determine whether long-term stimulation of the PCA muscle altered perceptual, acoustic, and aerodynamic parameters of voice. Two patients underwent implantation of a Medtronic Itrel II laryngeal pacemaker. Voice evaluation was performed before surgery and at monthly postoperative sessions with the pacemaker off. Months of PCA stimulation did not change perceptual descriptors of voice quality. Measures of fundamental frequency and intensity, upper and lower limits of the dynamic frequency and intensity range, and phonatory flow rates were largely unaltered. The results indicated that there was no effect of laryngeal pacing on voice.

KEY WORDS — bilateral vocal fold paralysis, electrical stimulation, laryngeal pacing, posterior cricoarytenoid muscle, voice.

INTRODUCTION

Bilateral laryngeal paralysis is a serious and often life-threatening medical condition. Injury to the recurrent laryngeal nerve arrests the vocal fold in the paramedian position, close to the midline. In the case of bilateral vocal fold paralysis (BVFP), the glottis is narrowed, severely restricting ventilation but allowing for near-normal voice production. Conventional surgical therapies for BVFP, such as arytenoidectomy and cordotomy, enlarge the airway at the expense of voice production, often leaving the patient with weak and breathy phonation and at risk for aspiration.[1-3]

Electrical pacing of the laryngeal abductor muscle is a promising experimental treatment for patients with BVFP.[4,5] Stimulation of the posterior cricoarytenoid (PCA) muscle, "paced" in synchrony with the inspiratory phase of respiration, restores glottal opening and allows ventilation. During noninspiratory phases of respiration, electrical stimulation is suspended, and the vocal folds passively relax to the midline to allow for voice production. Studies in animal subjects have confirmed that long-term electrical stimulation exhibits a protective effect on chronically denervated muscles of the larynx.[6,7] Specifically, contractility of the chronically denervated laryngeal abductor muscle was enhanced with electrical stimulation, and atrophy of type II muscle fibers was prevented and even reversed with 1 month of electrotherapy.

More recently, preliminary data on the chronically stimulated human larynx indicated that electrically stimulated abduction can restore ventilation through the mouth that is equal to or better than that through the patient's tracheotomy site.[5] Although the prospect of stimulated laryngeal opening to restore ventilation is the primary clinical objective in these patients, there has been no study of the effect of chronic laryngeal stimulation on voice production. The purpose of this investigation was to determine whether long-term electrical stimulation of the PCA muscle altered perceptual, acoustic, or aerodynamic parameters of the voice.

MATERIALS AND METHODS

Two patients underwent implantation of a Medtronic Itrel II laryngeal pacemaker (Fig 1). The device as it presently exists is not synchronized with inspiratory effort, but it was programmed to stimulate glottal opening at regular intervals, with the patients entraining their respiratory rate to that of the stimulus cycle. Figure 2 consists of endoscopic still frames of a bilaterally paralyzed larynx at rest and with stimulated glottal opening. The stimulus parameters were 2 to 5 V, 1-ms pulse duration, and 40 Hz. The device was set to stimulate glottal opening 10 times per minute, with a duty cycle of 2.5 seconds "on" and 3.5 seconds "off." Both patients began the study with a tracheotomy tube in place, which remained obturated during voice testing. The first patient was successfully decannulated 18 months into the study, and the second patient had the tracheotomy site obturated at all times.

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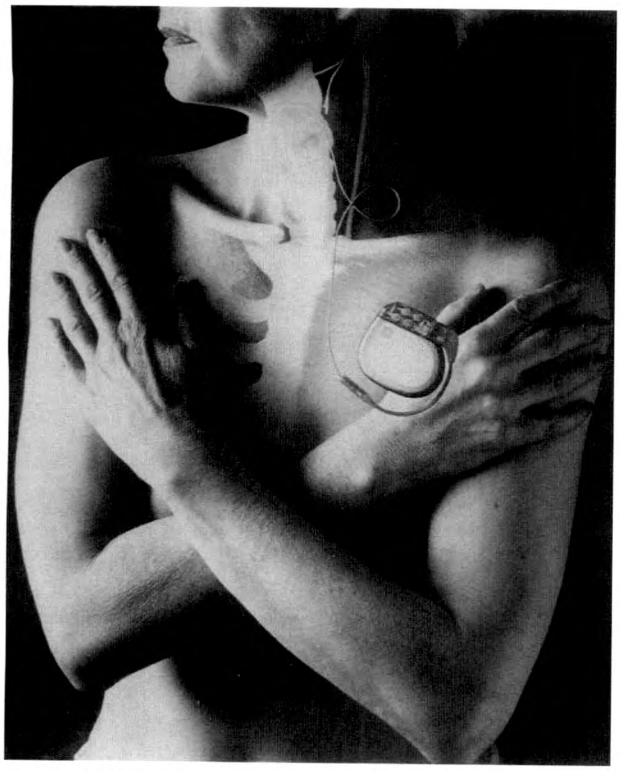

Fig 1. Schematic of laryngeal pacemaker as implanted in patient.

Voice evaluation was performed before surgery and at monthly postoperative visits. The laryngeal pacemaker was turned off for the testing sessions in order to compare the data to preoperative values. The GRBAS scale was used to assess perceptual changes in voice quality.[8] Aerodynamic and basic acoustic measures of voice were evaluated by means of the Nagashima Phonatory Function Analyzer and companion software for a personal computer. Specifically, measures of conversational fundamental frequency and intensity, and mean phonatory flow rates in reading were obtained during a 10-second portion of the Rainbow Passage. In order to document the dynamic frequency range, the patient was asked to phonate at the highest and lowest pitches possible. Similarly, the dynamic intensity range was assessed by having the patient vocalize at the loudest and softest levels possible. Three trials of each parameter of interest were averaged, and the data were displayed graphically.

In both patients, electromyography of the PCA and thyroarytenoid (TA) muscles performed both before and after operation confirmed reinnervation of these muscles. However, the vocal folds remained immobile because of the faulty or synkinetic nature of the reinnervation. It appeared problematic to discretely stimulate the abductor muscle in the presence of TA reinnervation. Current spread to the TA nerve supply was found to oppose the stimulated abduction of the PCA muscle. A 2.5-unit dose of botulinum toxin (Botox) into each vocal fold was given to patient 1 in order to eliminate antagonistic muscle activity. The injections were repeated every 2.5 to 3 months.

RESULTS

Electrical Stimulation of PCA Muscle Over 8- to 18-Month Period Did Not Alter Voice Quality. The GRBAS scale yields an overall rating or "grade" of voice quality based on evaluation of the descriptors "roughness," "breathiness," "asthenicity," and "strain." Each descriptor is assigned a number reflecting the degree of abnormal voice quality ranging from 0 (normal) to 3 (severe). Figure 3 is a graph of the overall grade assigned to patients at each follow-up visit by the senior speech-language pathologist on the laryngeal pacing team. Neither patient had a normal

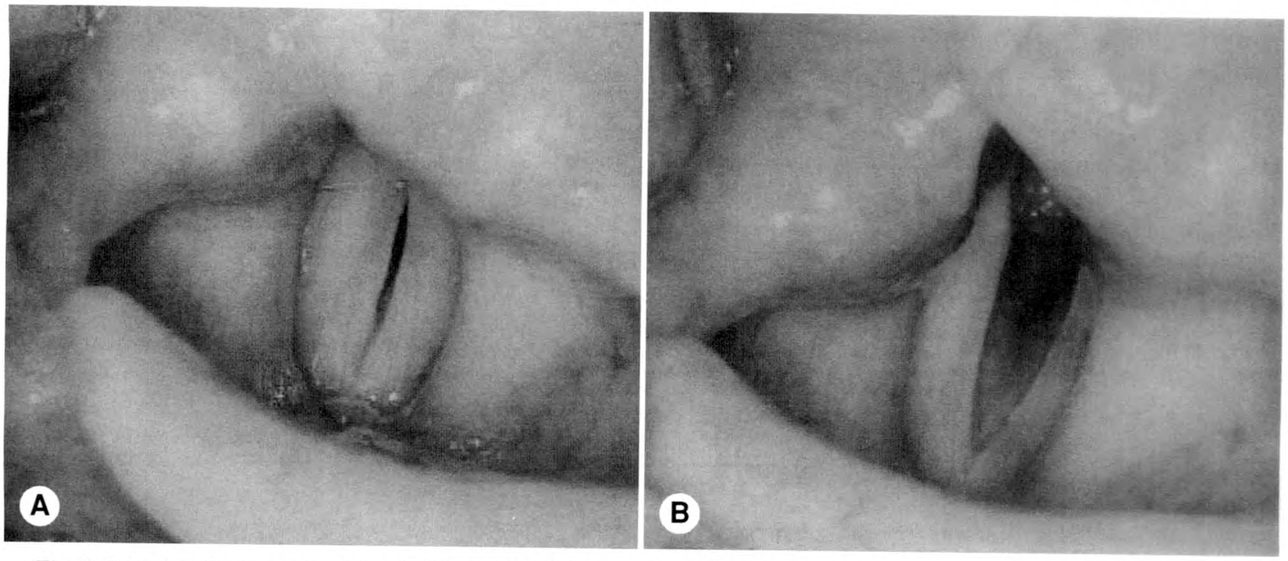

Fig 2. (Patient 1) Endoscopic views of patient with bilateral vocal fold paralysis **A**) at rest and **B**) with electrically stimulated glottal opening.

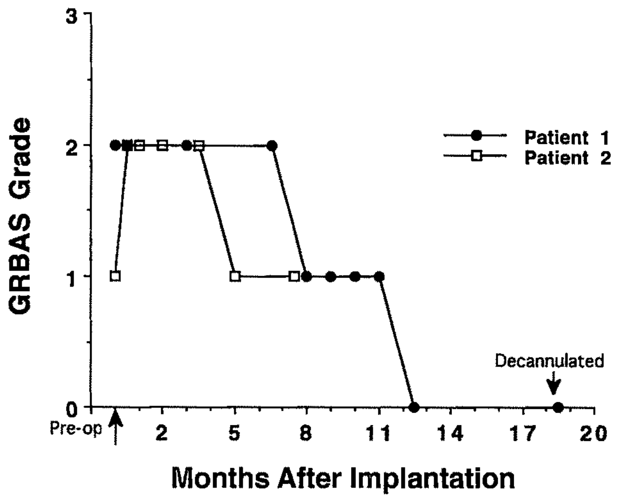

Fig 3. Changes in voice quality over course of laryngeal stimulation.

voice quality at the beginning of the study. Patient 1 presented with a moderately strained and rough voice quality, reflecting a compensatory hyperfunction adopted to allow a sufficiently loud and consistent voice. Her voice did not change after the implant surgery, nor did it change after 6 months of electrical pacing. In the seventh postoperative month, patient 1 began Botox therapy to eliminate antagonistic activity from the TA muscle. After Botox treatment, her voice quality improved, and with regular injections, it became normal. Patient 2 presented with a mildly breathy and asthenic voice quality before surgery. Immediately after implantation, the breathy quality of her voice increased, suggesting that there had been a temporary disturbance of the PCA muscle or its reinnervation during surgery. Over 4 months' time, the injury resolved and patient 2 returned to baseline voice quality. Her voice quality remained stable during the remaining months of electrical pacing.

Fundamental Frequency Was Not Affected by Laryngeal Stimulation. At each test session, the patients were asked to read a 10-second portion of the Rainbow Passage at a conversational pitch and loudness level. Figure 4 illustrates that the measures of fundamental frequency were remarkably stable over the course of the investigation. Long-term electrical pacing did not appear to alter this parameter in either patient.

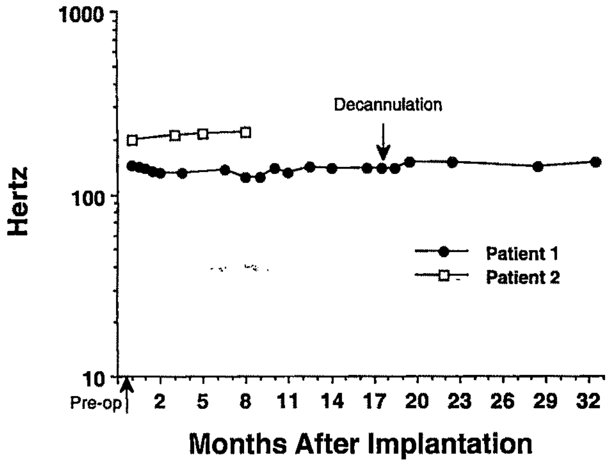

Fig 4. Measures of fundamental frequency obtained during period of muscle stimulation.

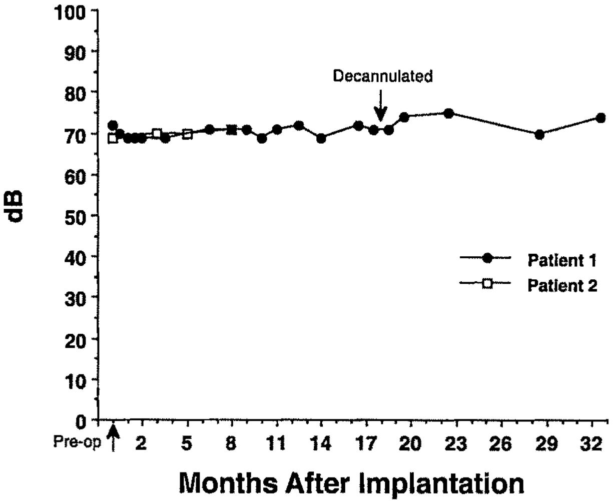

Fig 5. Null effect of chronic laryngeal stimulation on measures of conversational intensity.

Vocal Intensity Was Not Affected by Laryngeal Stimulation. Similarly, measures of sound pressure level were obtained during the reading exercise to assess whether chronic electrical pacing of the PCA muscle created any change in vocal loudness. Figure 5 confirms that use of the laryngeal pacemaker did not affect the level of conversational intensity over the 8- to 32-month period of study.

Dynamic Frequency and Intensity Ranges Were Maintained or Nominally Improved With Chronic PCA Stimulation. Figure 6 shows the frequency ranges for patients 1 and 2 prior to surgery and at

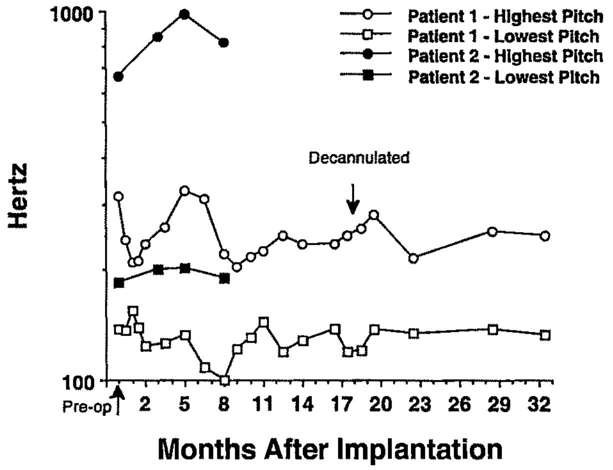

Fig 6. Changes in frequency range over course of laryngeal pacing.

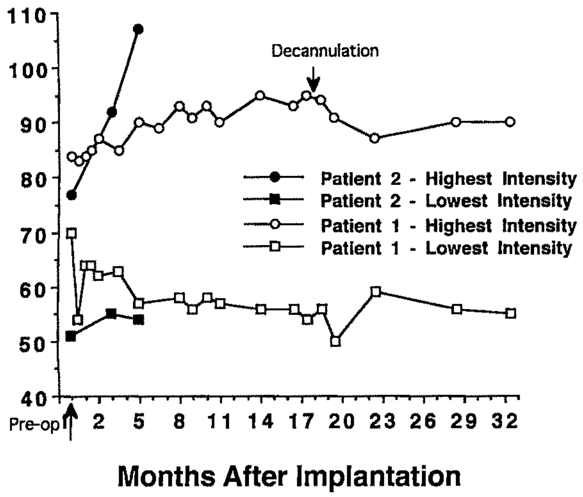

Fig 7. Measures of upper and lower limits of vocal intensity during period of chronic electrical stimulation.

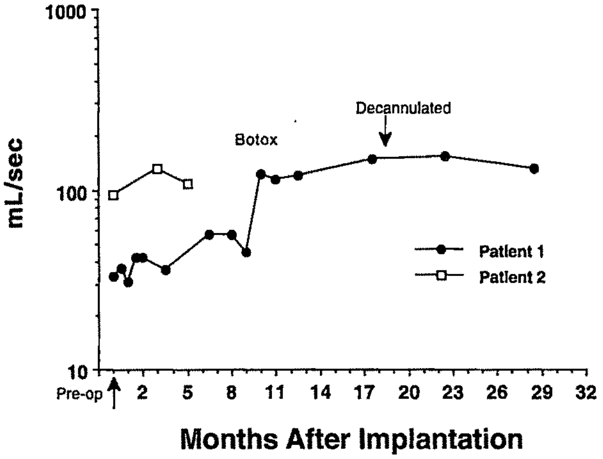

Fig 8. Measures of phonatory flow rates obtained with stimulator off to ascertain change in nonstimulated glottis. Flow rates were markedly increased after injection of Botox.

each postoperative visit. To obtain the end points for the range, each patient was asked to sustain the highest and lowest pitches possible for 1 second. Patient 1 consistently demonstrated a very limited frequency range, with little capacity to produce high-pitched sounds. This limitation was unchanged throughout 32 months of electrical pacing. Patient 2 had a remarkably stable lower limit, and appeared to demonstrate an improved ability to produce higher-pitched voice during the 8 months of laryngeal pacing.

With respect to vocal intensity, electrical stimulation may have enhanced the capacity to produce a greater range of sound pressure levels (Fig 7). Both patients 1 and 2 demonstrated the ability to produce a louder voice within the first few weeks of laryngeal pacing. Patient 2 demonstrated a dramatic 30-dB increase in intensity, and patient 1 showed a moderate, 9-dB gain. Over time, patient 1 also appeared to develop improved control of soft voice production, some 13 dB softer than the preoperative measures of minimum vocal intensity.

Airflow Rates in Speech Were Unchanged With Laryngeal Pacing, But Dramatically Increased After Botox Injection. Figure 8 shows that preoperative measures of flow rates during a 10-second sample of reading were essentially unchanged after months of PCA stimulation. There was, however, a remarkable increase in airflow during speech after patient 1 received 2.5 units of botulinum toxin in the TA muscle. The rapid ventilatory exchange during propositional speech quadrupled from 33 to 119 mL/s after Botox injection, and approached more-normal flow rates (140 to 200 mL/s).[9] Endoscopic examination of the vocal folds 1 month after injection confirmed a 2-mm increase in the static airway, which explained the dramatic increase in flow rates and suggested that there had been a slight lateral drift or relaxation of the vocal folds with Botox therapy.

DISCUSSION

Destructive surgical procedures performed to restore a viable airway have devastating effects on the voice. In contrast to other treatments for BVFP, laryngeal pacing restores ventilation with preservation of the voice. The present investigation determined that there was no adverse effect of long-term PCA stimulation on the voice. All vocal parameters measured were unchanged or maintained during laryngeal pacing over the 32-month course of study. Perceptual measures of voice quality determined no change with laryngeal stimulation. The conversational vocal intensity and fundamental frequency remained stable. The airflow rates in propositional speech were consistent, reflecting the fact that stimulation alone caused little or no change in the position of the nonstimulated glottis over time. The frequency and intensity ranges were maintained or slightly improved, suggesting an enhanced ability to vary pitch or loudness after weeks of pacing.

With respect to an improved dynamic frequency range, it is uncertain whether there was definitive gain, in view of the spread and limited number of data points for patient 2. On the other hand, there is a suggestion of a greater dynamic intensity range in both patients. We have two possible explanations as to why electrical stimulation might improve control of vocal loudness. In both patients, laryngeal stimulation led to a significant increase in the rate and volume of inspired air.[5] This gain in inspiratory capacity could result in greater subglottic pressure and generation of a louder voice. Alternatively, the enhanced ability to produce a loud voice could be related to a gradually increased PCA tonus consequent to long-term electrical stimulation. Previously, electrical stimulation has been shown to increase laryngeal muscle contractile strength and preserve muscle fiber integrity.[7] Perhaps an increased tone of the PCA muscle slightly changes the tensile force on the arytenoid cartilage, thereby favorably altering the dynamics of airflow through the posterior glottis and affording greater control of vocal intensity.

Botulinum toxin was found to be a useful adjunct to laryngeal pacing. Botox was effective in enhancing stimulated abduction. In both patients, before pacemaker implantation, electromyography confirmed synkinetic reinnervation of the laryngeal muscles. The position of the pacemaker electrode array was not optimal for isolated PCA stimulation. Stray current spread to nerve fibers that had reinnervated adductor muscles. This unwanted spread of current caused contraction of the laryngeal adductors, and dynamically opposed stimulated PCA abduction. Injection of botulinum toxin into the TA muscle eliminated much of the competitive response that antagonized PCA contraction, leaving stimulated abduction uncontested and allowing for maximum glottal opening. In addition, Botox induced a change in the position of the nonstimulated glottis by relaxing tension on the TA muscles. Bilateral muscle injections caused a subtle lateral drift of the vocal folds into a more favorable passive airway.

The laryngeal stimulation device as it presently exists is not synchronized with inspiration. It was programmed to stimulate glottal opening every 6 seconds, and the patients naturally entrained their inspiratory rhythm to that of the pacemaker. Of concern was that stimulation might lead to an interruption of voicing during propositional speech. Endoscopic examination of the larynx revealed that the patients could override the stimulated glottal opening, and there was no perceived change in voice quality.

Laryngeal pacing offers tremendous promise in a patient population for whom traditional surgical therapies are not optimal. Ongoing studies suggest that stimulated glottal opening can afford the patient ventilation at a level equal to or greater than that afforded by tracheotomy.[5] Given that there are no deleterious effects of long-term electrical stimulation of laryngeal muscles, and that the voice is maintained over time, laryngeal pacing has significant advantages over the current standard of care. Further investigation will determine whether laryngeal pacing becomes the treatment of choice for BVFP. Given the rapid advancements in biomedical technology, laryngeal pacing is likely to emerge as an accessible treatment method in the future.

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AIRFLOW RESISTANCE OF HEAT AND MOISTURE EXCHANGE FILTERS WITH AND WITHOUT A TRACHEOSTOMA VALVE

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Rehabilitation of laryngectomees has been furthered by the introduction of heat and moisture exchange (HME) filters, placed over a tracheostoma or on a tracheostoma valve (TSV). The airflow resistance of HME filters is an important factor with regard to the comfort of the patient. The goal of this study was to determine the airflow resistance (defined as the pressure drop over the device divided by the squared airflow through the device) of 4 commercially available HME filters with and without a TSV. The pressure drop over and the airflow through the devices were measured in vitro. Distinct differences among the devices were found. The mean airflow resistance of the HME filters ranged from 135 to 346 Pa • s^2/L^2, that of TSVs was between 66 and 297 Pa • s^2/L^2, and that of the combination was between 263 and 454 Pa • s^2/L^2. The Stom-Vent 2 HME filter and the Adeva Window TSV with an Adeva filter had the lowest airflow resistance of the devices measured in this study.

KEY WORDS — airflow resistance, heat and moisture exchange filter, laryngectomy, pressure drop, tracheostoma valve.

INTRODUCTION

One method of restoring speech after laryngectomy is with the aid of a tracheoesophageal shunt equipped with a shunt valve[1,2] such as the Blom-Singer valve,[3] the Provox valve,[4] or the Groningen button.[5] These are used regularly, in spite of their limited lifetimes.[6]

When the skin around the tracheostoma is sufficiently flat, mucus production is low, and endotracheal phonation pressure is limited, a tracheostoma valve (TSV) can be applied.[7-11] The patient can produce a spurt of air that closes the TSV, thus obtaining the capacity for hands-free speech.

To restore the nasal functions of laryngectomized patients, heat and moisture exchange (HME) filters have been introduced.[12,13] These HME filters were derived from comparable devices that are used in patients who are mechanically ventilated.[14-16] During expiration, an HME filter accumulates heat and moisture. During inhalation, the air is moisturized and warmed by the filter, thus preventing the tracheal tissue from drying out. This tracheal conditioning reduces both excessive mucus production and the occurrence of tracheal crusts. The filter can be attached to a TSV or applied directly by gluing it onto the skin like a TSV. A further advantage of an HME filter is that it offers an easy and efficient way to close the stoma manually.

Apart from the heat- and moisture-retaining capabilities, studied by Grolman et al[17] and Thomaschot et al,[15] airflow resistance is another factor determining the quality of HME filters.[17] Filters with a high airflow resistance require a high pressure to produce a certain airflow; thus, the patient must expend considerable energy in inhaling and exhaling. When these high pressures cannot be produced, a limited airflow will result that might cause problems in breathing.

The airflow resistance of HME filters has not been studied extensively. Only Grolman et al[17] have reported on airflow resistance. However, that article concentrates more on the economic aspects of HME filters than on airflow resistance. Pressure loss is determined only for 3 different airflow values and only during inhalation (Fig 1[17]); thus, the study is not accurate and extensive enough to determine airflow resistance behavior during inhalation and exhalation. Moreover, variations between different specimens of a device and the influence of time have not been studied.

The aim of this study was to give more insight into the airflow behavior of HME filters and of the combination of an HME filter and a TSV.

MATERIALS AND METHODS

Four commercially available HME filters, 2 commercially available TSVs, and 2 combinations of HME filters and TSVs were examined. The HME filters (Fig 2) were the Blom-Singer HumidiFilter (Inhealth Technologies, Carpinteria, California) in a

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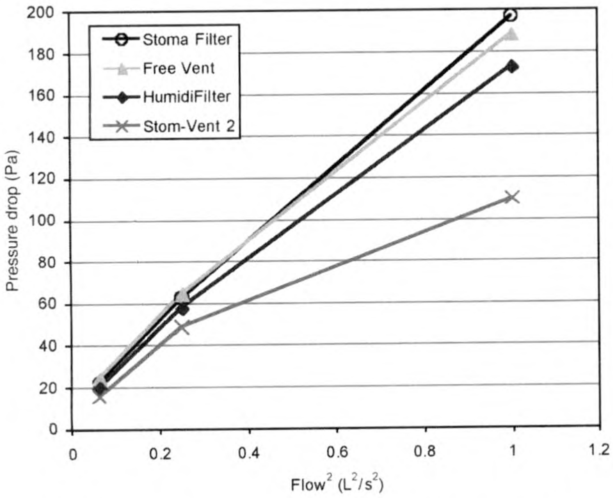

Fig 1. Relationship between pressure drop over (pascals) and squared airflow (liters squared per second squared) through heat and moisture exchange filters, as reported by Grolman et al.[17]

Blom-Singer HumidiFilter holder; the Provox Stoma Filter (Atos Medical AB, Hörby, Sweden) in a Provox Filter cassette; the Free Vent (Pharma Systems AB, Knivsta, Sweden); and the Stom-Vent 2 (Louis Gibeck AB, Upplands Väsby, Sweden). The TSVs (Fig 2) were the Blom-Singer ATV (adjustable tracheostoma valve, Inhealth Technologies) and the Adeva Window (Adeva Medical, Lübeck, Germany). The combinations were the Blom-Singer ATV with the HumidiFilter and the Adeva Window with the Adeva Filter.

Figure 3 shows schematically the test setup used to measure airflow resistance. Pressure drop was measured by HP pressure transducers (model 267BC, Hewlett Packard, Waltham, Massachusetts), and airflow was measured by a Lilly flowhead (Mercury Electronics, Glasgow, Scotland). Air was applied by mouth blowing. More details are given by Geertsema et al.[7] All 4 HME filters, the 2 TSVs (the Blom-Singer ATV is adjusted in the 2 extreme positions, marked 90 and 0), and the 2 combinations of HME filter and TSV were measured. Measurements were made in triplicate on 3 specimens of each of the devices. The breathing time per measurement was 10 seconds. The influence of breathing time was determined for 2 devices (Blom-Singer HumidiFilter and Provox Stoma Filter) by breathing through them for 1 hour, and then pressure drop and airflow measurements were made. The results are presented in graphs with the pressure drop plotted against the squared airflow.

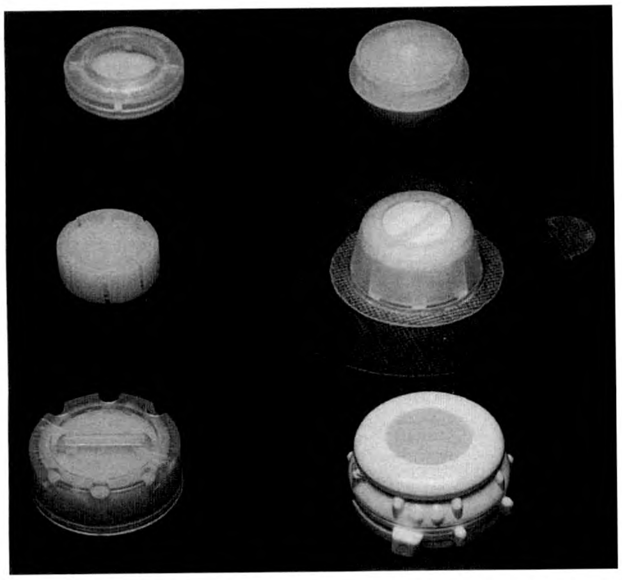

Fig 2. Blom-Singer HumidiFilter (upper left), Provox Stoma Filter (upper right), Free Vent (middle left), and Stom-Vent 2 (middle right) heat and moisture exchange filters. Blom-Singer ATV with HumidiFilter is at lower left, and Adeva Window TSV with Adeva Filter at lower right.

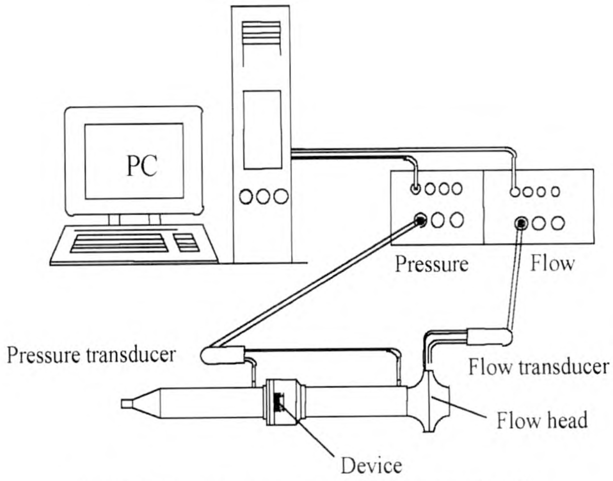

Fig 3. Schematic drawing of measurement setup.

RESULTS

In Fig 4, the pressure drop over a Stom-Vent 2 HME filter is plotted against the squared airflow. A linear relationship appears between pressure drop and squared airflow. All other HME filters and TSVs show a similar linear relationship. Table 1[17,18] depicts the mean airflow resistance, defined as the slope of the trend-line, of all the measured devices. Among the HME filters, the HumidiFilter shows the highest airflow resistance, and the Stom-Vent 2 the lowest. Among the TSVs, both with and without an HME filter, the Blom-Singer ATV-90 has the highest airflow resistance, followed by the Adeva Window and the Blom-Singer ATV-0. The airflow resistance of a combined HME filter and TSV is slightly higher than that of an HME filter alone.

Table 1 also depicts the range of measured results. This range is rather large for most devices, because a distinct difference among the 3 specimens was found. The maximum difference in airflow resistance

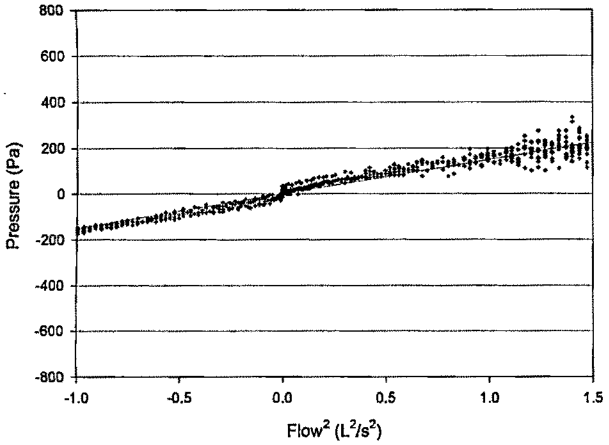

Fig 4. Relationship between pressure drop over (pascals) and squared airflow (liters squared per second squared) through Stom-Vent 2. Dots represent different measurements; line is result of linear regression.

between 2 specimens appeared to be 28%.

The airflow resistance changed little over time. After 1 hour of breathing, the airflow resistance changed by 2% at the most.

DISCUSSION

Several researchers have measured the airflow resistance of TSVs[7,8,18,19] and shunt valves,[4,5,20-24] but the airflow resistance of HME filters has not been measured extensively.

In addition to the closing behavior of TSVs and the HME capacity of HME filters, the airflow resistance of TSVs and HME filters is another important characteristic of these devices. The lower the airflow resistance, the more comfortable the patient will be. Breathing is a constantly repeated, and normally unconscious, activity. An increased airflow resistance caused by a TSV and an HME filter could make breathing effortful, and the problem would be compounded as the labored breathing became more conscious.

In healthy persons, the pressure drop and airflow resistance of the nasal, pharyngeal, and laryngeal cavities have been measured by several researchers.[25-29] In some cases, airflow resistance is defined as pressure drop divided by airflow, and not, as in the present article, by the *squared* airflow, which is a better way to describe airflow resistance.[30] Table 2[25-29] summarizes the airflow resistances that have been calculated from the results of these studies. Airflow resistance is defined as pressure drop divided by the *squared* airflow and is, when possible, calculated for a mean airflow of 0.5 L/s as representative of quiet breathing. The mean nasal airway (nasal passage, pharynx, and larynx) resistance ranged from 304 to 742 Pa • s^2/L^2 during inspiration and from 266 to 722 Pa • s^2/L^2 during expiration. The wide ranges and large SDs that have been reported are an indication that it is either very difficult to measure airflow resistance accurately or that large variations between subjects occur. Unfortunately, only a small number of subjects participated in the studies cited above.

TABLE 1. AIRFLOW RESISTANCE DERIVED FROM LINEAR REGRESSION LINES OF OUR MEASUREMENTS AND THOSE OF GROLMAN ET AL

	This Study (Pa • s^2/L^2)		*Grolman et al[17,18]*
Device	*Mean*	*Range*	*(Pa • s^2/L^2)*
Blom-Singer HumidiFilter	346	320-363	177
Provox Stoma Filter	286	275-294	201
Free Vent	166	150-194	193
Stom-Vent 2	135	122-166	115
Blom-Singer ATV in 0 position	66	65-67	43
Blom-Singer ATV in 90 position	297	289-301	172
Adeva Window	97	81-113	
Blom-Singer ATV in 0 position with HumidiFilter	263	241-293	
Blom-Singer ATV in 90 position with HumidiFilter	454	436-477	
Adeva Window with Adeva Filter	312	298-327	

The airflow resistance of the analyzed devices, again defined as pressure drop divided by the *squared* airflow, was derived from the measured data and summarized in Table 1 to compare it with the mean airflow resistance of the nasal airway. In general, the airflow resistance of the analyzed devices is lower than the reported mean airflow resistance of the nasal airway. Only when the data of Ferris et al[26] are used as a reference are the airflow resistance of the Blom-Singer ATV (adjusted in the most blocked position) with the HumidiFilter and that of the Blom-Singer HumidiFilter higher than the mean nasal airway resistance.

Some amount of airflow resistance is beneficial, because the pressure that builds up during exhalation keeps the lungs expanded, avoiding alveolar collapse.[31]

Our measurements in Fig 4 show that a quadratic relationship between the airflow and pressure drop of HME filters and TSVs characterizes airflow behavior very well. Breathing time has little influence. Differences between specimens do exist. In particular, the airflow characteristics of the Free Vent and the Stom-Vent 2 HME filters show a large variation, probably caused by variations in the thickness and pore size of the applied foams. Despite these variations, distinct differences exist among the 4 HME

TABLE 2. MEAN AIRFLOW RESISTANCE FOR INHALATION AND EXHALATION DERIVED FROM VARIOUS AIRFLOW RESISTANCE STUDIES

Structure	*Breathing*	*Cole et al*[25]	*Ferris et al*[26]	*McRae et al*[27]	*Schiratzki*[28]	*Wheatley et al*[29]
Nasal passages	Inhalation	256 ± 86	278 ± 311	582	NI	660 ± 60
	Exhalation	304 ± 94	238 ± 267	582	NI	640 ± 60
Oral cavity	Inhalation	NI	20 ± 22	NI	96 ± 38	168 ± 38
	Exhalation	NI	22 ± 25	NI	108 (44-236)	176 ± 34
Pharynx and larynx	Inhalation	232 ± 1,046	26 ± 29	NI	108 (24-320)	NI
	Exhalation	412 ± 1,694	28 ± 31	NI	34 (16-68)	NI
Nasal airway (nasal passage, pharynx, larynx) with mouth closed	Inhalation	488 ± 1,132	304 ± 1,140	742 (678-900)	NI	NI
	Exhalation	716 ± 1,188	266 ± 298	722 (662-832)	NI	NI
Oral airway (oral cavity, pharynx, larynx) with velum closed	Inhalation	NI	46 ± 52	NI	222 (108-452)	NI
	Exhalation	NI	50 ± 56	NI	150 (100-216)	NI
Airflow (L/s)*		NI	0.5	NI	0.5	0.5
No. of subjects		4	9	16	4-6	5

Data are Pa • s^2/L^2. Range or SD is given when available.

NI — not included in report cited.

*For which data are valid.

filters.

When we compare our data on the HumidiFilter, Free Vent, Stoma Filter, and Stom-Vent 2 with the data of Grolman et al[17] (Table 1), the same trends are found, but for 2 of the 4 HME filters, the airflow resistance values of Grolman et al clearly fall out of our range of data. Differences in the measuring setup could have caused the discrepancies in flow resistance. When pressure is measured at a point at which the flow profile is not fully developed, for instance, errors could occur. Also, the connection to the pressure transducer could disturb the flow profile and therefore cause errors. Another reason for discrepancies could be measuring only 1 specimen at a single moment. The description of the measurement setup and method of Grolman is not precise enough for us to check for these errors.

When we compare our data on the Blom-Singer ATV with the data of Grolman et al[18] (Table 1), the same trends are found, but our mean values are higher. Again, the reason could be a different measurement setup or method.

The valve position of the Blom-Singer ATV is a crucial factor in its airflow resistance. Airflow resistance can increase by a factor of more than 4 as a result of changing the valve position.

For TSVs with an HME filter, in general, airflow resistance is clearly dominated by the HME filter. The combination of the HME filter and TSV causes a slightly higher airflow resistance than does the use of only an HME filter, except for the 0 position of the Blom-Singer ATV with the HumidiFilter, which has a *lower* airflow resistance than a HumidiFilter alone. This is most probably caused by the fact that the HumidiFilter holder has a smaller airway passage than does the Blom-Singer ATV adjusted in the 0 position.

The airflow resistance data reported in this article, in combination with other characteristics such as the heat- and moisture-retaining capabilities of HME filters and the closing behavior of TSVs, will determine which device is best for a patient.

CONCLUSION

For HME filters and TSVs, there is a linear relationship between the pressure drop over the device and the squared airflow through the device.

The Stom-Vent 2 clearly has the lowest airflow resistance, less than half that of either the HumidiFilter or the Provox Stoma Filter. Airflow resistance varies considerably among the specimens, up to 28%.

In general, the Adeva Window has a lower airflow resistance than the Blom-Singer ATV. The airflow resistance of the Blom-Singer ATV depends highly on the valve position. The combination of an HME filter and a TSV has a slightly higher airflow resistance than an HME filter used alone.

In comparison with the reported mean airflow resistance of the nasal airway, most of the analyzed devices have a lower airflow resistance. However, the data on nasal airway resistance show a wide range, probably caused by external influences or by a large variation in patient characteristics.

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PRIMARY PAROTID GLAND HODGKIN'S LYMPHOMA

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Hodgkin's lymphoma with its primary manifestation in the parotid gland is an exceedingly rare entity and is not usually suspected in the initial evaluation of a parotid mass. Because it is not suspected, the results of fine-needle aspiration cytology are often misleading, and parotidectomy is needed for a definitive diagnosis. The most common subtype encountered is lymphocyte-predominant. The prognosis is favorable; the 5-year survival rate exceeds 90%. Treatment consists of chemotherapy, radiotherapy, or both. A case of primary parotid gland Hodgkin's lymphoma is presented along with a review of the literature and a discussion of the evaluation and management of this rare entity.

KEY WORDS — head, Hodgkin's lymphoma, neck, parotid gland, salivary gland.

INTRODUCTION

Lymphoma is a malignancy of lymphoid tissues that is broadly classified into 2 categories: Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). Within these broad categories, it is further subclassified. The vast majority of lymphomas occur within lymph nodes; however, lymphoma can present in an extranodal site. Approximately 25% to 30% of cases of NHL and 1% of cases of HL will present as extranodal disease.[1] The majority of extranodal lymphomas occur in the gastrointestinal tract, and the head and neck is the next most common site, accounting for 11% to 33% of all extranodal disease.[2] Within the head and neck, Waldeyer's ring is the most common site of origin; more than 50% of cases occur there. Lymphomas involving the parotid gland are uncommon; they only account for 0.6% to 5% of all neoplasms and/or tumorlike lesions involving this gland and 8% of all extranodal disease of the head and neck.[3] The vast majority of parotid gland lymphomas are of the non-Hodgkin's type. Involvement of the parotid gland by HL is exceedingly rare. Reported here is a case of primary parotid gland HL presenting as a parotid mass, along with a review of the literature and a discussion of the evaluation and management of this entity.

CASE REPORT

A 41-year-old man was referred to the otolaryngology clinic for evaluation of a right cervicofacial mass. Questioning revealed that the mass had been present for about 2 years, with an increase in size noted over the preceding 6 to 8 months. The patient denied fever, night sweats, or weight loss. Physical examination revealed a 3 × 2-cm, firm, nontender, nonmobile mass involving the right parotid gland. Also noted, on the right, was a small (1 cm) mass overlying the mastoid process; it was thought to be a postauricular lymph node. The facial nerve was intact (grade I of VI). The rest of the findings of the head and neck examination were within normal limits. A fine-needle aspiration (FNA) biopsy was performed; the findings were interpreted as those of a probable Warthin's tumor. Magnetic resonance imaging and axial computed tomographic (CT) scans were obtained. The CT scan demonstrated a 3-cm, enhanc-

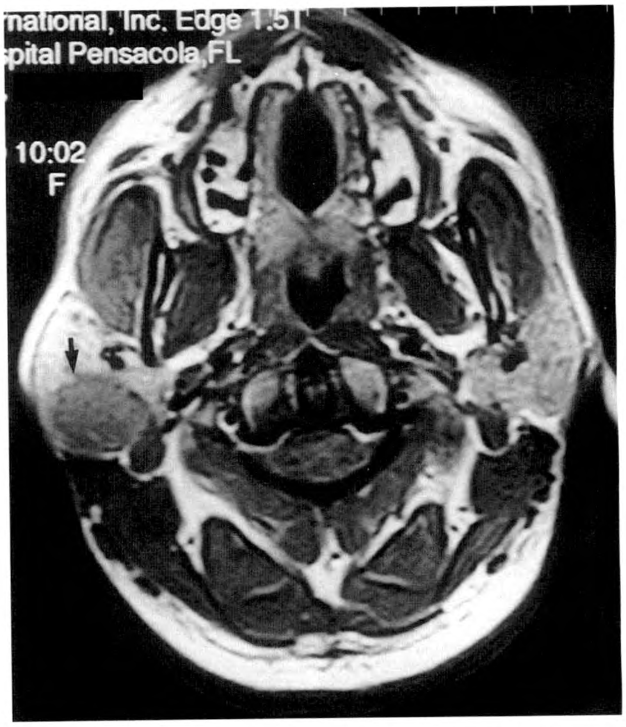

Fig 1. Magnetic resonance image of primary parotid lymphoma (arrow).

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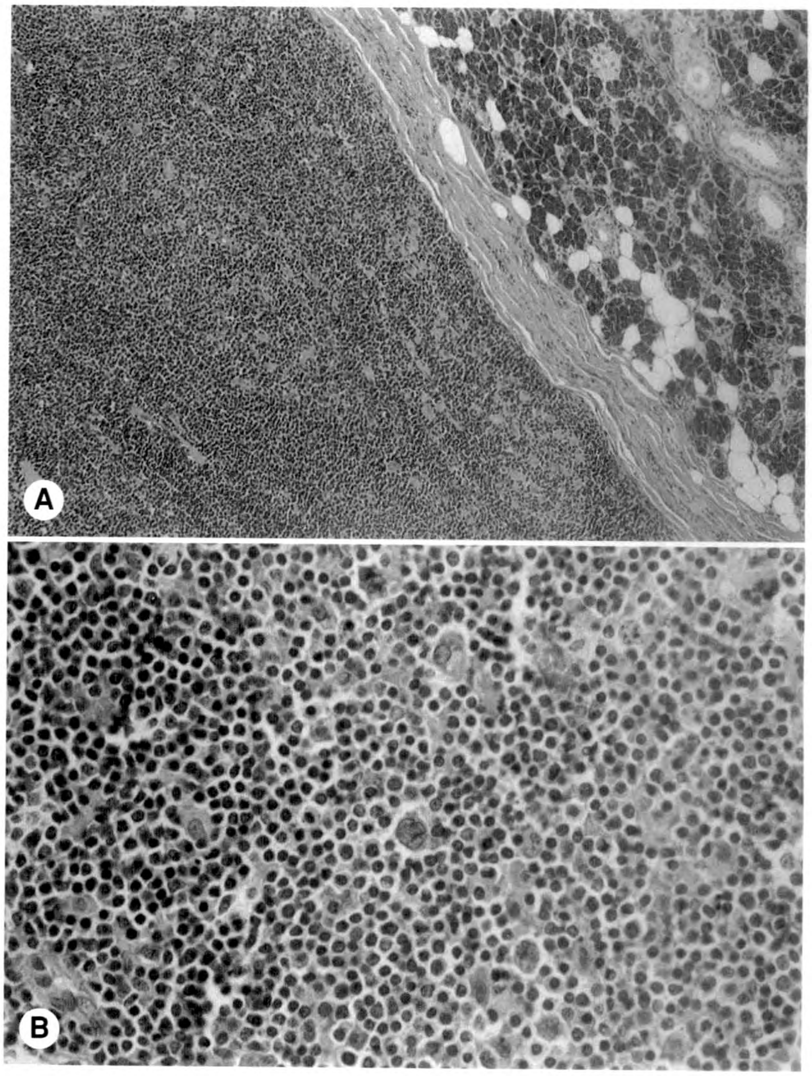

Fig 2. Intraparotid lymph nodes effaced by lymphocyte-predominant Hodgkin's disease. **A**) Original ×100. **B**) Original ×400.

ing intraparotid mass on the right, and magnetic resonance imaging identified a 3.5 × 2.9 × 2-cm right intraparotid mass with its center in the superficial lobe (Fig 1). Because of the size of the mass, surgery was recommended, although lymphoma was not suspected. Informed consent was obtained, and the patient underwent right superficial parotidectomy with preservation of the facial nerve. During the operation, we discovered that what we thought to be a postauricular lymph node was actually part of the parotid mass. A portion of the mass did extend into the deep lobe.

The initial histopathologic examination was suspicious for HL, so the case was referred to the Armed Forces Institute of Pathology for further evaluation. Their final diagnosis was lymphocyte-predominant HL (Fig 2).

Further workup included CT scans of the chest, abdomen, and pelvis, along with a gallium scan, all of which were negative for systemic involvement. The patient was staged IE. After evaluation by medi-

TABLE 1. CRITERIA USED TO DEFINE PRIMARY PAROTID GLAND LYMPHOMA

Any lymphoma originating in either glandular lymph nodes or glandular parenchyma, regardless of its association with autoimmune disease or its subsequent stage; must be initial clinical manifestation of lymphoma
Any lymphoma originating in glandular parenchyma as initial clinical manifestation of lymphoma
Any lymphoma not associated with autoimmune disease, benign lymphoepithelial lesion, or myoepithelial sialadenitis
Any lymphoma originating in glandular parenchyma not associated with autoimmune disease
Any lymphoma originating either in glandular parenchyma or in glandular lymph node with invasion of parenchyma, in absence of detectable disease outside of parotid gland
Any lymphoma originating in glandular parenchyma in absence of extraparotid involvement at time of diagnosis

Information from references 3, 5-9.

TABLE 2. RESULTS OF LITERATURE REVIEW

Subtypes	*No.*	*%*
Lymphocyte-predominant	13	40
Nodular sclerosis	7	22
Mixed cellularity	5	16
Lymphocyte-depleted	2	6
Not available	5	16
Total cases of primary parotid gland Hodgkin's lymphoma	32	

Includes present case and references 5, 9, 14, 15, 17-31. Of 19 patients, 15 (79%) were male and 4 (21%) were female. Average age was 49.6 years.

cal and radiation oncologists, the treatment plan consisted of 4 cycles of doxorubicin hydrochloride, bleomycin sulfate, vinblastine sulfate, and dacarbazine followed by a 2,400-cGy dose of radiation to the head and neck. The patient is currently free of disease after 12 months of follow-up.

DISCUSSION

Hodgkin's lymphoma has a yearly incidence of 3 per 100,000; in comparison, NHL has a yearly incidence of 16 per 100,000.[4] Hodgkin's lymphoma has a bimodal age distribution, with the first peak occurring during the second decade of life and the second peak during the fifth decade of life. In contrast, the incidence of NHL increases with advancing age. The average age for patients presenting with HL is 32 years, as compared with 52 years for NHL.[1]

Risk factors for the development of lymphoma include immunodeficiency (congenital or acquired), history of radiation exposure, immunosuppression, Sjögren's syndrome (40 to 44 times higher risk than for general population), rheumatoid arthritis, celiac disease, and Hashimoto's thyroiditis.[1]

Hodgkin's lymphoma is classified into the following subtypes (frequency of occurrence in parentheses): nodular sclerosis (70%), mixed cellularity (25%), lymphocyte-predominant (3%), and lymphocyte depletion (2%).[4] The author's patient had the lymphocyte-predominant subtype.

Histopathologically, the classic appearance of HL includes the presence of Reed-Sternberg (R-S) cells in a background of reactive lymphocytes, plasma cells, and macrophages, with a disruption of the nodal architecture. The various subtypes have their own unique distinguishing characteristics; however, a discussion of these characteristics is beyond the scope of this article.

Parotid gland lymphomas are classified as extranodal and can originate within glandular lymph nodes or glandular parenchyma. They are also categorized into primary or secondary; however, in the literature, there are numerous criteria used to define primary parotid gland lymphoma (Table 1[3,5-9]). The author used the first of the criteria listed in Table 1 to define primary parotid gland lymphoma; the present case, accordingly, is classified as such.

Lymphomas that originate in the parotid gland usually present as an asymptomatic mass, and FNA has tremendous diagnostic potential in the initial evaluation of a parotid mass by providing a preliminary diagnosis. With this initial diagnosis, the surgeon can counsel the patient with some certainty, plan for the expected procedure, and, in the case of a definitive diagnosis of lymphoma, obviate the need for surgery and its associated morbidity. In reviewing the literature, the diagnostic accuracy rate of FNA cytology for parotid masses ranges from 85% to 93.5%[10-14]; however, in the author's experience, it is only 72%. A definitive diagnosis of lymphoma, especially NHL, can be established on cytologic examination with the use of adjunctive testing such as flow cytometry and immunophenotyping.[15] In the case of HL, flow cytometry is used to rule out NHL and support a morphological diagnosis of HL; however, a definitive diagnosis requires the identification of either R-S cells or R-S cell variants in conjunction with immunocytochemical analysis.[15,16] A recent study demonstrated an accuracy rate of 66% for diagnosing HL on FNA cytology; however, because of its inherent difficulty, subtyping is not routinely performed on cytologic specimens.[16] An example of the difficulty of diagnosing HL on cytologic examination is evident in the current case and another case,[14] both of which were misdiagnosed as probable Warthin's tumors.

Primary parotid gland HL is exceedingly rare; only 32 cases have been reported in the English-language literature, and the largest single study consisted of only 4 cases (Table 2[5,9,14,15,17-31]). Lymphocyte-predominant seems to be the most common subtype affecting the parotid gland; in contrast, nodular sclerosis is commonly diagnosed in *classic* nodal HL.

Parotid gland lymphomas tend to have a more favorable prognosis then do lymphomas in general.[5,32] Although primary parotid gland HL is a rarity, it tends to have a better prognosis than its NHL counterpart: a 5-year survival rate of 91%, as compared with 51% to 80% for NHL.[3,9,17-31] Accounting for this better prognosis may be the fact that parotid gland HL tends to be more localized than parotid gland NHL at the time of diagnosis. As expected, stage I or II disease has a better prognosis than stage III or IV disease. In addition, with regard to HL, the stage of disease and the age of the patient are more important than the subtype in terms of prognosis.[16]

Finally, if a definitive diagnosis of lymphoma cannot be established from FNA cytology, then parotidectomy with preservation of the facial nerve is recommended. If, during the course of the surgery, the diagnosis of lymphoma is in doubt, then frozen-section analysis should be performed and the surgery modified accordingly. Once lymphoma is established as the diagnosis, the initial staging consists of CT scans of the chest, abdomen, and pelvis. Next, the patient should be referred to both medical and radiation oncologists for definitive treatment, which consists of chemotherapy alone or in combination with radiotherapy.

CONCLUSION

Primary parotid HL is a rare entity that is not usually suspected in the evaluation of a parotid mass. The most common subtype encountered is lymphocyte-predominant. The prognosis is more favorable than that of its nodal counterpart; its 5-year survival rate exceeds 90%. Treatment consists of chemotherapy, radiotherapy, or both.

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EDITOR'S NOTE

This is an uncommon event. It has been verified at the Armed Forces Institute of Pathology and also by John G. Batsakis, MD.

Brian F. McCabe, MD
Editor

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LIMITATIONS TO MOBILIZING THE INTRAPETROUS CAROTID ARTERY

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The irregular and complex osteology of the bony skull base houses the intrapetrous internal carotid artery (ICA), which represents a potential obstacle to the complete extirpation of benign skull base lesions. This 2-part study 1) investigated the cadaveric basis for the mobilization of the intrapetrous ICA and 2) correlated the cadaveric anatomic findings with the authors' clinical experience. We conclude that the ICA can be mobilized relatively safely. The degree of mobility achieved directly relates to the surgical approach and exposure. Limited mobility is achieved when an anterior petrosal approach is used with various neurosurgical procedures. Conversely, transcochlear and infratemporal approaches allow for optimal ICA translation. The safety of ICA mobilization is documented by the low complication rate in our series.

KEY WORDS — internal carotid artery, mobilization, skull base surgery.

INTRODUCTION

The intrapetrous carotid artery creates a potential obstacle during extirpation of petrous apex and skull base lesions. Its course through the petrous bone's carotid canal leads from posterolateral to anteromedial, and vital structures, including cranial nerves, vessels, the otic capsule contents, and the brain, abound in its immediate vicinity. Mobilization of the artery in its petrous segment may permit complete excision of benign skull base lesions; however, mobilization represents a formidable challenge, given the artery's relative inaccessibility. Moreover, the extent of mobilization is limited, and the risks of complications with potentially devastating sequelae are very real. The skull base surgeon must carefully weigh the merits of complete tumor extirpation via internal carotid artery (ICA) mobilization, with its attendant limitations and risks, versus subtotal lesion removal with the carotid artery remaining intact.

Since Lempert's[1] seminal paper addressing petrous apicectomy, numerous authors have detailed the vagaries of the ICA. Various anatomic studies and resultant classification schemata of the vessel's course have emerged.

Detailed knowledge of the ICA's course is paramount in skull base surgery. This understanding, coupled with advances in skull base surgical techniques over the past quarter-century, has allowed ICA mobilization for tumor extirpation to become a reality. The aim of this study is to evaluate the surgical anatomy of the ICA and the limitations encountered in mobilizing the vessel during dissection of cadaveric head preparations. We then correlate these findings with our own clinical experience in 60 cases of benign skull base tumor extirpation by various surgical approaches.

MATERIALS AND METHODS

Anatomic. Ten cadaveric heads were prepared for study. Fresh specimens were used in 5 preparations with gel injection, and 5 specimens were studied in formalin-fixed heads. For macroscopic investigation, after injection of the fresh head preparations, initial exposure was obtained by a frontolateral transzygomatic intracranial-extradural approach. Subsequently, brain tissue from the middle and anterior fossae was selectively removed with careful preservation of the underlying neural and vascular elements. Beginning with the vertebral arteries, the vessels at the base of the skull and the neck, followed by the common carotid artery or the external and internal carotid arteries, were dissected independently for approximately 2 cm and cannulated with an appropriate-diameter catheter. Tap water was used to mechanically flush the clots from the system.

Arterial injection was performed with a preparation of 40 mL of 3110 room temperature vulcanized silicone rubber from Dow-Corning mixed with a double volume (80 mL) of 5% Dow-Corning fluid thinner. In the venous system injection, the ratio was 1:1, and 80 mL was used of both silicone solution and fluid thinner. Water-soluble Crayola powder paint (Artista powder paint, Binney & Smith, Easton, Pennsylvania) was mixed with the silicone solution

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and rubber until the desired color was obtained. Slow catalyst (Catalyst S, Dow-Corning) was added to the silicone at a ratio of 1:10 immediately before injection, resulting in the slow solidification of the silicone within 3 to 4 hours, after which the head was refrigerated. The injection sites were clamped immediately after preparation, and the clamps were left in place for 12 to 16 hours to prevent spillage.

Subsequent posterior petrosectomy was performed with removal of the auricle, external auditory canal, tympanic membrane, and ossicular chain. The extratemporal facial nerve was identified, and the mandible was cut at its angle and disarticulated from the glenoid fossa.

The carotid artery was identified in all surgical specimens, and the horizontal and vertical segments were measured. The horizontal segment was readily identified with an anterior petrosectomy dissection. In this study, the cochlea was removed after the measurements were noted and the vertical segment was made visible from both a subtemporal exposure and a posterior transpetrosal view.

Operative. Between 1990 and 1998, 60 patients underwent skull base surgical procedures that required identification and manipulation of tumors juxtaposed to the petrous carotid artery. The patients (37 women and 23 men; ages, 16 to 74 years) had 31 meningiomas, 18 glomus tumors, 6 neuromas, 3 chondromas, and 2 desmoplastic fibromas. We excluded patients who were undergoing anterior petrosectomy for basilar aneurysms, petrosectomy with craniotomy for clival chordomas, or temporal bone resection for malignant tumors.

The following surgical approaches were used: 1) modified Dolenc frontotemporal craniotomy, orbitozygomatic osteotomy, and anterior petrosectomy,[2] 2) subtemporal transcavernous anterior petrosectomy,[3] 3) subtemporal transzygomatic combined anterior-posterior petrosectomy,[4] 4) infratemporal fossa approach,[5] 5) subtotal petrosectomy with translabyrinthine-transcochlear dissection,[6-8] 6) Fukushima's modified transpetrosal exposure,[9,10] and 7) extreme lateral transjugular approach.[11] All patients underwent preoperative electrophysiological and neuroradiographic studies. Balloon test occlusion was performed only in cases in which complete tumor encasement was noted with narrowing of the ICA. In all cases, cranial nerve function was monitored during surgery, as were median nerve somatosensory-evoked potentials, used for monitoring brain stem function.

RESULTS

Anatomic. The petrous carotid canal was dehiscent and naturally exposed the carotid artery on the floor of the middle fossa in 8 (40%) of the 20 specimens. In 6 preparations (30%), a thin lamina of bone was found that could be readily removed with gentle pick dissection.

The carotid artery was invested in a variably thick areolar tissue intimately juxtaposed to the periosteum of the carotid canal in all cases. In 3 specimens, periosteal penetrating branches were observed along the horizontal segment. Venous plexus elements were variably noted, although the anterior portion of the horizontal segment posterior to the cavernous sinus contained the largest confluence of vessels. Sympathetic neural elements were also identified.

The greater superficial petrosal nerve (GSPN) and lesser superficial petrosal nerve (LSPN) lie lateral to the horizontal petrous carotid artery. The GSPN was identified and followed to the facial hiatus in 17 specimens. The LSPN was identified in 14 specimens. More difficulty was encountered in identifying both nerves in the preserved preparations. Anterolateral to the artery, the eustachian tube and tensor tympani muscle were identified. The carotid canal was dehiscent at the genu in 8 preparations in which the common wall between the eustachian tube and the carotid canal was not complete. Moreover, the thin lamina of bone that was found in 9 additional specimens was readily pierced with a blunt dissector by means of minimal pressure along the floor of the protympanic space. The caroticotympanic artery entered the middle ear through a foramen, abutting the vertical segment in 6 preparations. No evidence of a persistent stapedial artery was encountered in any dissection.

The horizontal segment, measured from the posterior portion of the foramen lacerum to the genu at the cochlea at which it turns into its vertical segment, extended an average length of 20 mm (range, 15 to 25 mm). The vertical segment, measured from the skull base to the upper portion of the genu, extended an average length of 10.2 mm (range, 6 to 15 mm). The diameter of the horizontal segment bony canal averaged 5.6 mm (range, 3 to 9 mm), and the diameter in the bony vertical segment averaged 5.2 mm (range, 4 to 8 mm).

The vidian or pterygoid artery may arise from the horizontal petrous carotid artery or the internal maxillary artery. It was identified arising from the horizontal petrous carotid artery in 4 preparations.

The posterior portion of the gasserian ganglion, which gives rise to the V2 and V3 segments, was found to lie just along the anterior margin of the carotid artery. To demonstrate the full course of the

OPERATIVE RESULTS BASED ON TUMOR TYPE

Tumor Type (n)	*Complete Excision*	*Subtotal Excision*	*Residual Gross Tumor*
Glomus tumor (18)	11	3	4
Neuroma (6)	4	2	0
Desmoplastic fibroma (2)	2	0	0
Chondroma (3)	2	1	0
Meningioma (31)	11	15	5

carotid artery, V3 needed to be mobilized or sacrificed in all cases.

The cochlea, located posterior or posterosuperior to the carotid genu, lay an average of 3.8 mm (range, 3 to 4.5 mm) deep to the floor of the middle fossa. In all cases, the carotid canal intimately effaced the basal turn. Of note, the facial hiatus for the transmission of the GSPN covered the genu of the ICA in all cases just anterior and lateral to the cochlea.

Operative. Tumor was found to intimately efface the ICA in 17 patients (28%). In 14 patients (23%), an ill-defined plane in the dissection field did not allow for clear separation of the ICA-tumor interface. In the remaining 29 patients (48%), the carotid artery was easily separated from the tumor.

The following surgical approaches, combined in some cases, were used: modified frontotemporal craniotomy with orbitozygomatic osteotomy and anterior petrosectomy (22 cases); subtemporal transcavernous, anterior petrosectomy dissection (7 cases); subtemporal transzygomatic combined anterior-posterior petrosectomy (12 cases); infratemporal fossa dissections (11 cases); subtotal petrosectomy with translabyrinthine-transcochlear exposure (5 cases); modified transpetrosal exposure (7 cases); and an extreme lateral transjugular approach (2 cases).

Anterior petrosectomy with craniotomy was performed in 36 cases, in which total gross tumor removal was achieved in 24 cases, subtotal removal was accomplished in 8 cases, and preplanned cytoreductive surgery was performed in 4 cases. When anterior and posterior petrosectomy were combined and the cochleovestibular end organ was preserved, no further ICA mobilization was possible other than that obtained with an anterior dissection alone.

However, infratemporal approaches, used in 11 cases, did allow for increased mobilization of the carotid artery. A dense cartilaginous-bony ring was encountered at the entrance of the vertical segment of the artery at the skull base, and the ICA could be mobilized anteriorly once the fibrous banding around the artery was segregated from the periosteum of the carotid canal. In general, full mobility was possible once the artery was freed at the basal turn of the cochlea and exposed from the upper cervical region to the precavernous segment.

In the 5 cases in which a transcochlear approach was used with a subtotal petrosectomy, very adequate ventral superior mobilization was accomplished. This procedure allowed for full exposure of the artery and, thus, among all procedures, optimized mobilization and the opportunity for circumferential tumor extirpation.

For the patients with meningioma, total gross tumor removal was noted in 23 cases. Subtotal resection was accomplished in 5 cases with gross tumor present along the nerves within the cavernous sinus and carotid artery. Cytoreductive surgery was performed in 3 patients to decompress the brain stem. No attempt was made to isolate and clearly dissect the ICA.

Glomus tumors were completely resected in 11 patients. In contrast to both neuromas and meningiomas, the glomus tumors invaded the carotid canal and adventitial layer. In 3 patients, tumor was subtotally resected, and in 4 patients, gross tumor was left encircling the ICA; all 7 patients underwent postoperative radiotherapy.

Four of 6 neuromas were excised completely. Both neuromas of the apex (nerve V) and jugular foramen were dissected cleanly from the ICA, as were both desmoplastic fibromas approached via subtemporal anterior petrosectomy. Two of 3 chondromas were totally resected, and 1 was subtotally removed to preserve vagus nerve function; however, carotid involvement was not extensive.

In cases of artery effacement by tumor, a conservative stance was adopted, accepting residual tumor mass in situ rather than performing complete excision with resultant disruption of physiologic circulation. This approach was applied to 7 of 18 glomus tumors, 2 of 6 neuromas, 1 of 3 chondromas, and 20 of 31 meningiomas. The surgical results are summarized in the Table.

There were no deaths in this series. The ICA was anatomically preserved in 58 cases. In 2 patients, the artery was sacrificed during tumor removal with no untoward postoperative sequelae. The ICA was violated in 2 cases, requiring suture control of bleeding during surgery. There were no postoperative cerebrovascular accidents due to manipulation of the carotid artery, nor did any patient sustain blindness as a result of compromise of the ophthalmic artery.

DISCUSSION

The petrous segment of the ICA originates at the

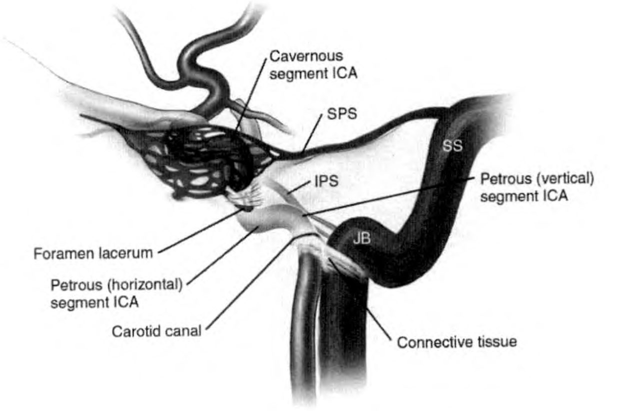

Fig 1. Lateral view of intrapetrous carotid artery in relation to bony and venous structures from its entry into carotid canal to foramen lacerum. ICA — internal carotid artery; SPS — superior petrosal sinus; IPS — inferior petrosal sinus; SS — sigmoid sinus; JB — jugular bulb. (Printed with permission from Mayfield Clinic, Cincinnati, Ohio.)

entrance to the carotid canal, anterior to the jugular foramen and posteromedial to the vaginal process of the tympanic bone, and terminates at the posterior edge of foramen lacerum (Fig 1). In the cadavers, the horizontal segment was found to be an average of 20 mm long, and the vertical segment averaged 10.2 mm long, consistent with prior studies.[12]

Two branches may arise from the intrapetrous carotid artery: the caroticotympanic artery and the vidian or pterygoid artery. The former, which arises from the vertical segment of the carotid artery, supplies the tympanic cavity, whereas the latter, which commonly arises from the internal maxillary artery, may originate at the horizontal petrous segment and subsequently enter the pterygoid canal. These arteries are variable in origin and location.[12]

The intrapetrous carotid artery is intimately related to the trigeminal ganglion and nerve (Fig 2). Usually, the portion of the ganglion from which the maxillary and mandibular divisions arise rests directly above the anterior margin of the carotid artery. Full ICA exposure thereby mandates sacrifice of V3.

The cochlea is located posterior or posterosuperior to the carotid genu, lying an average of 3.8 mm deep to the middle fossa floor. Given the intimate relationship between the basal turn and the genu, great potential exists for injury to the superior aspect of the basal turn during exposure of the artery. An anterolateral approach should, therefore, be used in mobilizing the carotid artery to better identify the cochlea.

The need for sacrificing the carotid artery is dependent on tumor type and behavior. Paragangliomas grow slowly, but progressively invade the carotid canal periosteum with subsequent involvement of the arterial wall. In contrast, neurinomas may erode into the carotid canal, compressing and circumferentially surrounding the perivascular connective tissue, but without direct invasion. Benign lesions that are not invading the carotid artery are the best candidates for complete tumor extirpation with carotid artery mobilization.

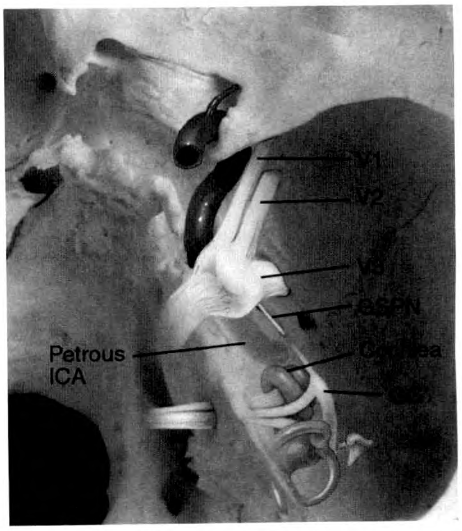

Fig 2. Superior oblique view of petrous ridge depicts intrapetrous carotid artery in relation to trigeminal nerve and cochlea. V1 — ophthalmic division of trigeminal nerve; V2 — maxillary division of trigeminal nerve; V3 — mandibular division of trigeminal nerve; GSPN — greater superficial petrosal nerve; GG — geniculate ganglion. (Printed with permission from Mayfield Clinic.)

The question arises then, whether carotid artery sacrifice, with its attendant risks of ischemic neurologic deficits, is truly the management of choice in selected skull base lesions. Issues to consider include the nature of the tumor (benign or malignant), the natural history and survival rate of the tumor, and whether resection of tumor remaining in other regions of the skull base is possible after successful carotid artery management. In the case of malignancies, complete tumor extirpation is the clear goal, unless sheer extent renders the lesion unresectable, in which case palliative treatment becomes the priority. However, the management of benign lesions is less clear, because despite the desire for complete excision from a surgeon's perspective, the risk of major neurologic deficits or life-threatening hemorrhage outweighs the benefit of total resection of a benign tumor. In fact, Souliere et al[13] advocate that carotid artery resection is never warranted in the case of benign skull base disease, and in all of their patients, superb local tumor control has been achieved via subtotal resection with postoperative radiotherapy administered when appropriate. Their method entails

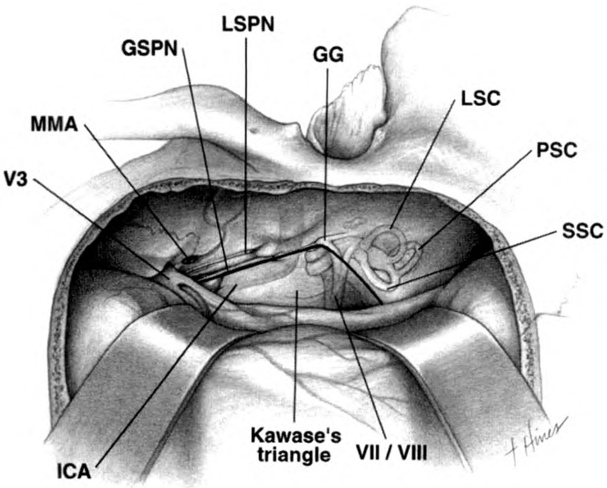

Fig 3. View of petrous ridge via middle fossa approach shows Kawase's triangle. Kawase's triangle is bounded by GSPN laterally, petrous ridge medially, and arcuate eminence (superior semicircular canal [SSC]) at its base. LSPN — lesser superficial petrosal nerve; LSC — lateral semicircular canal; PSC — posterior semicircular canal; MMA — middle meningeal artery; VII/VIII — seventh and eighth cranial nerves. (From Tew JM, van Loveren HR, Keller JT. Brain tumors. Philadelphia, Pa: WB Saunders, 2001. [Atlas of operative microneurosurgery; vol 2, p 49, figure 4.2]. Reprinted with permission.)

identification of a surgical plane between the carotid artery adventitia and tumor; if this plane is absent, residual tumor is allowed to remain on the carotid artery and is subsequently cauterized. Conversely, Sekhar et al[14,15] and Zane et al[16] demonstrated that preoperative occlusion of the carotid artery with subsequent sacrifice is a relatively safe and viable management option in patients with benign lesions.

However, carotid artery reconstruction offers a means to achieve complete tumor extirpation with carotid sacrifice while preserving physiologic circulation. Revascularization may be seriously considered for cases of known significant carotid involvement in patients in whom preoperative balloon test occlusion fails or shows little reserve cerebral blood flow. However, some surgeons support use of the procedure in any case necessitating sacrifice of the carotid artery.[17] The type of revascularization procedure chosen depends on several factors, including the site of the tumor and the outcome of the balloon test occlusion and cerebral blood flow studies. The acceptable revascularization options include bypass from the superficial temporal artery or occipital artery to the middle cerebral artery, long vein grafts from the cervical arteries to the middle cerebral artery, and short vein grafts from the petrous carotid artery to the supraclinoid carotid artery. The last is ideal for bypass of a tumor-encased cavernous carotid artery and necessitates exposure of the petrous carotid artery. Although revascularization has a favorable impact on the restoration of stable cerebral circulation, the procedure is not without disadvantages and risks. Prolonged operative time increases the risks of general anesthesia, and the transfusion rate climbs to 3 times that of cases in which revascularization is avoided.[18] Also, despite impeccable surgical technique, immediate or delayed occlusion of the graft and breakdown of the site of anastomosis, with resultant hemorrhage, represent distinct possibilities.

Various approaches exist for skull base, petroclival, and cerebellopontine angle lesions that require exposure and/or mobilization of the intrapetrous carotid artery for extirpation.[19-22] Transpetrosal techniques provide maximum exposure of the ICA. Synonymous nomenclature includes the subtemporal-transpetrosal, extended middle fossa, middle fossa transpetrous, transcochlear, and extended translabyrinthine approaches, just to mention a few. The unwieldy list of names describing various transpetrosal techniques has been simplified by Miller et al,[23] who recognize just 2 subtypes: anterior and posterior petrosectomies.

Cass et al[24] built upon the petrosectomy concept in their description of a total petrosectomy approach incorporating features of both transcochlear and subtemporal-infratemporal fossa methods. In their series, the carotid artery was exposed in its cervical through petrous segments and mobilized anteriorly. In any case mandating ICA mobilization via an anterior petrosectomy approach, section of the third division of the trigeminal nerve, the GSPN, and the middle meningeal artery is necessary for adequate transposition of the vessel. In our operative series, mere mobilization of V3 or traction on the gasserian ganglion rarely permitted more than 5 mm of mobility, whether anteriorly or laterally. This limitation is due to the relative proximity of the two fixation points: the gasserian ganglion and the cochlea.

The opening of Kawase's triangle to the posterior fossa dura allows a very limited degree of mobility laterally in dissection medial to the artery (Fig 3).[25] The boundaries of Kawase's triangle are the GSPN laterally, the petrous ridge medially, and the arcuate eminence at the base. If preservation of hearing is intended, mobility is limited once again by the position of the geniculate ganglion and the basal turn of the cochlea.

The transcochlear approach affords the surgeon excellent exposure and a flat angle of approach to the clivus without the need for cerebellar or temporal lobe retraction. It is ideal for midline intracranial lesions, intradural clival tumors, and cerebellopontine angle disease arising anterior to the internal auditory canal. This approach optimizes ICA mobilization by providing excellent ventral and superior relocation during extirpation of lesions. Permanent loss of hearing and transient facial palsy represent the main disadvantages.

The infratemporal fossa approach described by Fisch and Pillsbury[5] allows the surgeon to expose the ICA. Removal of the bony and cartilaginous eustachian tube exposes the horizontal portion of the carotid canal extending to the foramen lacerum. Complete exposure is effected by removing the tensor tympani muscle. Separation of the carotid canal periosteum from the dense fibrocartilaginous ring surrounding the artery at the entrance to the skull base permits mobilization of the ICA. Mobilization of the artery inferiorly and anteriorly, coupled with facial nerve rerouting, affords outstanding exposure of the entire petrous apex.

CONCLUSION

Since the time of Lempert's[1] discussion of temporal bone and ICA anatomy concerning apicectomy, various surgical approaches have evolved to address disease juxtaposed to the ICA. The ICA is indeed accessible, and given the strong nature of its investing adventitia, manipulation and mobilization of the vessel are possible. However, mobilization of the ICA is limited, mainly because of its irregular course through the complex anatomy of the temporal bone and the numerous adjacent vital structures. In fact, maximization of mobilization requires sacrifice of the middle meningeal artery, the superior petrosal sinus, the mandibular branch of the trigeminal nerve, and the cochlea, as well as repositioning of the facial nerve in selected posterior approaches. It is our opinion that mobilization of the carotid artery represents a viable option for exposure and extirpation of benign skull base lesions. In agreement with Souliere et al,[13] we do not advocate sacrifice of the ICA in these cases, because of the potential formidable morbidity of this procedure in comparison with the lesser morbidity of subtotal resection of a benign lesion with maintenance of the physiologic circulation.

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EFFECTS OF FREQUENCY, INSTRUMENTAL FAMILY, AND COCHLEAR IMPLANT TYPE ON TIMBRE RECOGNITION AND APPRAISAL

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The purpose of this study was to compare postlingually deafened cochlear implant recipients and normal-hearing adults on timbre (tone quality) recognition and appraisal of 8 musical instruments representing 3 frequency ranges and 4 instrumental families. The implant recipients were significantly less accurate than the normal-hearing adults on timbre recognition. The implant recipients gave significantly poorer ratings than did the normal-hearing adults to those instruments played in the higher frequency range and to those from the string family. The timbre measures were weakly correlated with speech perception measures, but were significantly correlated with 3 cognitive measures of sequential processing.

KEY WORDS — cochlear implant, music, normal hearing, timbre, tone quality.

INTRODUCTION

Because cochlear implants have been designed primarily to assist in reception of spoken communication, it is not surprising that most technical advances and research have addressed improved speech perception and communication competency. However, given attainment of reasonable transmission of speech sounds in current devices and the emergence of advanced processing strategies, musical enjoyment seems a logical objective with regard to potential implant benefit. Because music is such a pervasive art form and common acoustic event in everyday life, it is likely that implant recipients will be exposed to musical sounds regularly, and they are likely to be interested in active listening. Indeed, after the perception of speech, the appreciation of music is the aspiration next most commonly expressed by users of cochlear implants.[1] Unfortunately, many implant recipients characterize the sound of music after implantation as unpleasant or difficult to follow.[2]

This report focuses on perception of one aspect of music, timbre. Timbre, represented by the spectral envelope, is "that attribute of auditory sensation in terms of which a listener can judge that two sounds similarly presented and having the same loudness and pitch are dissimilar."[3(p45)] Timbre assists one in recognizing which musical instrument has produced a sound, and contributes to auditory scene analysis as one listens to complex musical ensembles such as orchestras. Further, the unique tone qualities of various instruments is an important aspect of the aesthetic experience of music. Therefore, the extent to which a cochlear implant transmits unique and rich timbral representation is not insignificant with regard to listener satisfaction.

Current-day implants are more effective at providing temporal information than at providing pitch resolution, but the more subtle features of the spectral envelope are not faithfully represented with typical speech processing strategies.[4] Those few extant studies regarding timbre recognition suggest that implant recipients using various devices (Clarion,[4] Ineraid,[5] Med-El,[6] and Nucleus[7]) were less accurate than normal-hearing adults were in open-set recognition of synthetic representations or recordings of 4 or 5 different musical instruments. However, their accuracy improved in closed-set testing conditions, particularly after brief training.[5-7]

Although it is enjoyable to be able to identify which musical instrument is being heard, recognition is not required in order to derive pleasure from music. Possibly of greater importance with regard to implant satisfaction is the extent to which the sound is pleasing. Gfeller and Lansing[8] found that 8 implant recipients using Ineraid implants (analog coding strategy) rated a larger percentage of musical in-

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struments as pleasant or beautiful than did 10 recipients of the Nucleus CI22M device (F0F1F2 feature extractor). The analog strategy of the Ineraid may have transmitted a more complete or natural representation of the spectral envelope than did the feature extraction strategy of the Nucleus. However, since that time, many new coding strategies have been developed; consequently, these earlier results might not represent the experiences of many recent implant recipients. Although 2 studies comparing timbral ratings of normal-hearing adults with those of implant recipients with newer devices or strategies (Med-El,[6] Clarion[4]) continue to indicate that some instruments sounded significantly more pleasant to normal-hearing listeners than to implant recipients, those studies did not permit a contrast between different processing strategies.

The existing body of research regarding timbral perception by implant users is quite limited in scope and in depth. Several of the prior studies provide data from relatively small numbers of implant recipients (eg, N = 8,[6,7] N = 16,[5] N = 18[8]), which can make generalization difficult, given the well-documented variability in performance of implant recipients on a variety of tasks. Further, past studies have seldom examined timbral recognition in concert with subject characteristics (eg, hearing history, age) that could influence performance. It is also possible that timbral features may be more or less effectively transmitted by various coding strategies. Finally, it is important to address parameters in the musical stimulus, as well as the implant, as a source of variation in perceptual outcomes. Most extant timbre studies with implant recipients do not provide in-depth descriptions of the timbral stimuli used (eg, frequency range, instrumental family categories), nor have they tested as independent variables specific stimulus properties that may have an impact on recognition or ratings of sound quality.

Therefore, the purposes of this study were 1) to compare postlingually deafened implant recipients with normal-hearing adults on timbre recognition and appraisal; 2) to test the effects of specific structural parameters (frequency ranges, instrumental families) of music on recognition and appraisal; 3) to compare outcomes of recipients who use 4 different devices; and 4) to examine the relationships between subject characteristics (eg, age, length of implant use) and timbre recognition and appraisal.

MATERIALS AND METHODS

Subjects. The subjects included normal-hearing and postlingually deafened cochlear implant recipients. The normal-hearing adults were recruited through an advertisement and had to pass a hearing screening in order to participate. None of the normal-hearing subjects were professional musicians, as determined by administration of the Iowa Musical Background Questionnaire, which is described by Gfeller et al.[2] Briefly, the questionnaire includes 6 items regarding formal music training and experiences prior to hearing loss, and requires respondents to list the number of years of training or experience in specific categories (eg, instrumental lessons; participation in band, orchestra, or chorus; classes in music theory or music appreciation) at the elementary, high school, college, and postcollegiate levels.

The implant recipients were regular patients in our clinical research center and had at least 12 months of experience in using one of the following devices: Clarion, Ineraid, Ineraid implant with a Med-El processor, or Nucleus. Only 1 of the implant recipients was a professional musician as determined by administration of the Iowa Musical Background Questionnaire.[2] The implant recipients were tested during their annual audiology visits to the clinic, during which they took speech recognition and electrophysiological tests, as well as a music perception test. Because of limited time available for testing, not all subjects were able to complete all 3 tests in this study. Therefore, more specific information regarding the sample will be described in conjunction with the results for each specific test.

Sound Stimuli. The timbral stimuli used in the 3 tests described below included 8 different musical instruments that are commonly known to non-musicians, representing 3 different fundamental frequency ranges (low, 131 to 262 Hz; medium, 262 to 534 Hz; high, 534 to 1,068 Hz) and representing 4 different instrumental families based on principles of sound production (brass, woodwind, pitched percussion, strings). Given the dependent variable of recognition, less commonly known instruments that could have represented a particular frequency range for a given instrumental family (eg, the viola, a mid-range string instrument, which is often confused with the violin) were not included. The trumpet (medium) and trombone (low) represented the brass family. The flute (high), clarinet (medium), and saxophone (low) represented the woodwind family. The violin (high) and cello (low) represented the string instruments. Each of these instruments was presented in its characteristic frequency range. Pitched percussion was represented by the piano, which was played in 2 different frequency ranges (medium and high, both of which are equally characteristic of the piano).

Past studies of timbre with normal-hearing adults have often used synthesized or highly controlled samples of isolated pitches in timbre testing. Multidimensional scaling, which is valuable in investigating the contribution of physical attributes to the quality of sound, requires extensive test time beyond that available during the annual visits of our patient population and, more important, does not address sound appraisal, a key issue in this investigation.[4] Furthermore, although isolated and synthesized tones have the advantage of greater experimental control, it is difficult to extrapolate the findings from such isolated stimuli to contextualized experiences of "real-life" music listening (ie, ecological validity).[9-11] Even trained musicians can have difficulty correctly identifying musical instruments from isolated and synthetic representations.[12] Consequently, recordings of real instruments playing a standardized connected melodic sequence, which include transients that are important cues for recognition, were used in this study.

The specific structural features of the stimuli were selected as follows. The melodic pattern played by each instrument was composed specifically for use in these tests. It consisted of a 7-note sequence of equal-duration notes (a quarter note is equal to 100 beats per second) that included intervalic changes (stepwise movement and skips) within the basic framework of a C-major scale. Eight professional musicians recorded the melodic pattern on their respective instruments at the University of Iowa Electronic Studios using a single Neuman microphone approximately 5 feet from the test instrument at an approximate angle of 10°. The microphone was a Mackie 1402-VLZ Mixer with phantom power. Sound was recorded onto a Panasonic SV-3700 DAT Recorder with Sony PDP-124 DAT tapes that were played back and digitally transferred to a PowerMac 8500/120 computer Digidesign Audiomedia III sound card with Digidesign Sound Designer II. All sounds were extracted into individual aiff mono files with a sample rate of 44.1 kHz (16-bit sound).

These timbral stimuli were used in 3 different tasks: 1) Timbre Recognition, that is, identification of the instrument being played by sound alone; 2) Timbre Appraisal Test A: General Appraisal, or overall pleasantness of the sound; and 3) Timbre Appraisal Test B: Qualitative Ratings for Three Dimensions of Timbre.[13,14] For all subjects, test stimuli were presented via an Apple Computer (model M340-9) with a Sony touch screen (model CPD-2000SF), with the sound files presented through Yamaha external speakers (model YST-M15). The test was administered in sound field in a quiet room, at 70 dB sound pressure level. The implant recipients were allowed to adjust their processor to the most comfortable level of loudness.

Timbre Recognition. Before the timbre recognition test, the computer touch screen displayed a grid picturing 16 commonly heard musical instruments and the name of each instrument. The subjects were instructed to touch the picture of each musical instrument that they knew by sound before they became deaf. Although instruments were chosen that were considered well known to the general public, musical training and experiences differ considerably across the general population. Therefore, it is possible that a person may be unfamiliar with one or more of the instruments. Those instruments that were not known by an individual before deafness, as determined through this preliminary step, were excluded from the analysis of data in order to eliminate items missed because of lack of prior familiarity as opposed to those items missed because of perceptual error.

After completion of 2 practice items, stimuli based on each instrument were presented 3 times in random order. After each melody was played, the grid with pictures and names of 16 different instruments (uneven match) appeared on the touch screen, and the individual selected the instrument that he or she believed produced the sound just heard. The computer automatically recorded all responses. No feedback on accuracy was provided during or after testing. The test results were reported as percent correct of those instruments known by sound before hearing loss.

Timbre Appraisal Test A: General Appraisal. Although some individual differences in preference for particular instrumental sounds are to be expected even for normal-hearing listeners, comparisons of ratings by implant recipients with those of normal-hearing listeners help identify whether the quality of sound heard by implant recipients is generally less pleasant than what is heard by normal-hearing listeners, or whether there are particular types of sounds that are particularly pleasant or disagreeable for implant recipients.

In Timbre Appraisal Test A, a subject was asked to appraise the overall pleasantness of each item by touching the point along a 100-mm visual analog scale (anchored with the bipolar adjectives of "dislike very much" at 0 and "like very much" at 100) that represented their opinion. The subjects were told in the preliminary instructions that there was no right or wrong answer, that they should give their most honest appraisal of the sound quality, and that they should feel free to use the entire range of the 100-mm scale. Each item was played twice in random order. No information about the instrument being played was provided during the task; therefore, the appraisal was based on the sound only, as opposed to possible preconceived biases about particular instruments. The computer automatically recorded each response.

Timbre Appraisal Test B: Qualitative Ratings for Three Dimensions of Timbre. Factorial investigations on verbal reports of musical sound quality indicate that 88% of variance can be accounted for with 3 scales: dull-brilliant (or sharp), compact-scattered, and full-empty.[13,14] In prior experiments,[14] normal-hearing listeners have judged sounds with more low-frequency energy as more dull (on a continuum of dull to sharp) in quality, whereas sounds with more high-frequency energy were judged as more sharp (brilliant) in quality. Normal-hearing listeners have rated those sounds with more noise as sounding more scattered (on a continuum of compact to scattered). Sounds more rich in harmonics tend to be judged as more full (on a continuum of empty to full).

Variety in instrumental timbre (eg, the more characteristically hollow sound of the clarinet, versus the very rich and deep sound of a cello) contributes to the novelty and beauty that listeners seek in music; thus, for one sound to be judged as more "empty" than another is not inherently undesirable.[4] However, implant recipients have often described the quality of musical instruments as sounding more thin or shrill than their recollection of how a particular instrument sounded before hearing loss. Furthermore, considerable "noise" (ie, scattered) in a musical signal is usually undesirable, other than for special stylistic effects (eg, brief use of a growling tone quality to highlight a particular phrase in the "blues" genre, or purposeful distortion in "heavy metal" rock music). Thus, if implant recipients tend to rate sounds as less full, less brilliant, or more scattered than do normal-hearing adults, these data would suggest that the implant transmits an uncharacteristic or inferior quality in contrast to what is heard through a normal ear.[2,4] Therefore, these 3 bipolar adjectives were used as the anchors for 3 visual analog scales of 100 mm each to measure these 3 dimensions of sound quality of each timbral item.

Each instrument was presented twice and in random order. After each item, the subjects registered their opinions by touching a point on the visual analog scale for each of the 3 bipolar adjectives. Subjects were told that there is no right or wrong answer, that they should give their most honest appraisal of the sound quality, and that they should feel free to use the entire range of the 100-mm scale. The computer automatically recorded each response. No information about the instrument being played was provided.

Speech Perception Measures. Timbre perception scores were correlated with the following speech perception measures: The Iowa Version of the NU-6 test,[15] the Iowa Consonant Test[16] in noise and without noise (sound only), the Iowa Sentence Test,[16] and the Iowa Vowel Test.[17]

Cognitive Test Measures. Timbral perception scores were correlated with the following cognitive measures: the Visual Monitoring Task (VMT) and the Sequence Completion Test (SCT). The VMT is a laboratory test developed at the University of Iowa that tests attention, reaction time, continuous performance, and working memory. Numbers are presented on a visual display at a rate of either 1/s (VMT1) or 2/s (VMT2); subjects respond when the current number and the 2 preceding numbers represent an even-odd-even sequence. The VMT has been a strong predictor of implant recipient success in both speech recognition and music perception and appraisal tasks regardless of the implant design used.[4,18] The SCT is based on the work of Simon and Kotovsky.[19] Subjects are presented a series of letters that represent a progressive sequence, with the last 4 letters missing. The subject must complete the sequence. Only moderately correlated with the VMT, this test of sequence identification has also been shown to predict implant benefit.

RESULTS

Timbre Recognition. Twenty normal-hearing adults (15 female, 5 male) ranging in age from 18 to 72 years (mean, 29.7 years) took the Timbre Recognition test. Fifty-one implant recipients (30 female, 21 male) who were tested for timbre recognition ranged in age from 24 to 81 years (mean, 55.06 years). At the time of testing, they had between 12 and 152 months of implant use (mean, 80.8 months) with one of the following devices: Clarion (CIS strategy, N = 24); Ineraid (analog strategy, N = 3); Ineraid implant with a Med-El processor (CIS strategy, N = 11); and Nucleus (spectral peak [SPEAK] strategy; CI22M, N = 12; CI24M, N = 1).

The normal-hearing adults achieved a mean score of 90.9% correct (SD, 9.88%; range, 67% to 100%) for timbre recognition; implant recipients achieved a mean score of 46.55% correct (SD, 23.17%; range, 11% to 100%). A *t*-test revealed that this difference was statistically significant at the .001 confidence level. The instrument most often recognized by the implant recipients was the piano. The error patterns differed between implant recipients and normal-hearing adults. The normal-hearing adults made fewer errors, but when they were in error, they generally confused one instrument with another instrument within the same instrument family (eg, mistaking a violin for a cello, or a clarinet for a saxophone). The implanted subjects showed diffuse error patterns,

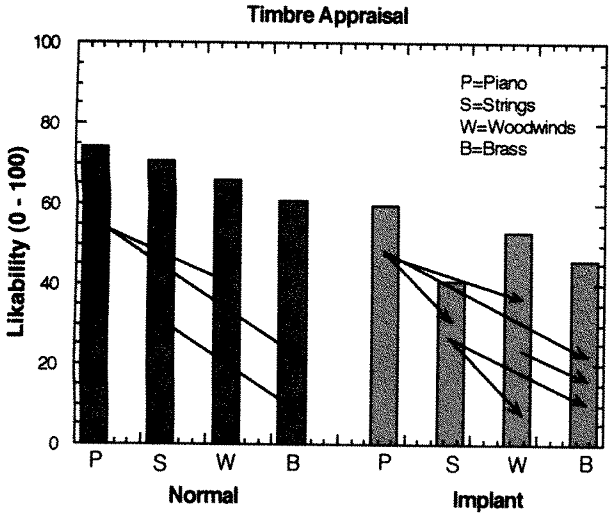

Fig 1. Timbre appraisal by instrument family. Arrows indicate significant differences between instrument families of <.03 to .0001. (For normal-hearing subjects, $p < .0001$ for brass versus piano; $p < .001$ for brass versus strings; and $p < .003$ for piano versus woodwinds. For cochlear implant users, $p < .0001$ for brass versus piano, piano versus strings, and strings versus woodwinds; $p < .001$ for brass versus woodwinds; $p < .0014$ for piano versus woodwinds; and $p < .03$ for brass versus strings.)

often mistaking instruments from different families for the target item.

The recognition scores were examined by device. The mean scores were as follows: Clarion, 50.67% (SD, 24.62%); Ineraid, 51.33% (SD, 4.04%); Med-El, 53.27% (SD, 23.43%); and Nucleus, 32.15% (SD, 17.59%). The *t*-tests reveal significantly greater accuracy on the part of the Clarion recipients ($p < .01$) and the Med-El recipients ($p < .02$) as compared with the Nucleus recipients. Correlations between age and length of implant use and percent correct revealed weak negative correlations for age ($r = -0.12$) and length of implant use ($r = -0.20$).

Timbre Appraisal Test A: General Appraisal. Eleven of the normal-hearing adults (8 female, 3 male) who were also tested for timbre recognition took Timbre Appraisal Test A for general appraisal. They ranged in age from 18 to 56 years (mean, 22.55 years). Forty-eight implant recipients (41 who also took the Timbre Recognition Test) took Timbre Appraisal Test A. They ranged in age from 24 to 81 years (mean, 55.29 years), and they had used their implants between 36 and 152 months (mean, 84.37 months). They used the following devices: Clarion (N = 24); Ineraid (N = 3); Ineraid device with Med-El processor (N = 11); and Nucleus CI22M, SPEAK strategy (N = 10).

An analysis of variance revealed a significant interaction for group (normals versus implant users) by instrumental family ($p < .0008$) and interactions for instrumental family by frequency range (low, medium, high; $p < .0001$). The *t*-tests indicate the following sources of differences: significantly higher ratings by normals than by implant recipients for instruments in the string family ($p < .04$); significantly higher ratings by normals for the lower string instrument (cello) compared with the higher string instrument (violin; $p <. 001$); and significantly higher ratings by implant recipients for lower-frequency woodwind instruments (low woodwind versus high woodwind, $p < .0001$; medium woodwind versus high woodwind, $p < .03$) and string instruments (low versus high strings, $p < .0001$). Figure 1 shows the mean ratings by group (implant versus normal) and family (brass, woodwind, piano, string). The internal consistency of responses for Timbre Appraisal Test A was calculated to be .74.

With regard to spectral characteristics of instrumental families, when members of a family (eg, violin and cello in the string family, or trumpet and trombone in the brass family) are large- and small-scale versions of one another, the spectral characteristics exhibited by an instrument in a family will be observed at higher frequencies for the smaller instruments of a family.[19] However, in the case of the 3 woodwind instruments used, there are more structural differences in the instrumental design, including the type of reed (air reed for flute, and mechanical reed for clarinet and saxophone) and the material from which the body of the instrument is made (eg, silver, wood, brass). Therefore, ratings for individual instruments, as well as for family groups, are worthy of note. Table 1 shows the mean appraisal scores for the individual instruments. For the implant re-

TABLE 1. TIMBRE APPRAISAL TEST A: GENERAL APPRAISAL

	Normal-Hearing Adults (N = 11)			*Cochlear Implant Recipients (N = 48)*		
	Low	*Medium*	*High*	*Low*	*Medium*	*High*
Brass	61.03 ± 20.67	60.55 ± 15.15		47.30 ± 22.44	44.68 ± 21.98	
Woodwind	68.69 ± 12.29	63.45 ± 10.44	67.11 ± 22.61	59.63 ± 20.55	53.25 ± 22.34	46.13 ± 25.48
Piano		74.89 ± 9.31	69.06 ± 11.32		62.83 ± 21.02	56.79 ± 22.39
String	82.85 ± 10.79		61.75 ± 22.05	53.98 ± 20.88		27.78 ± 20.95
Mean	70.85	66.30	65.97	53.64	53.59	43.57

Data are mean ± SD; 0 — "dislike very much"; 100 — "like very much." "Low," "medium," and "high" refer to frequency range.

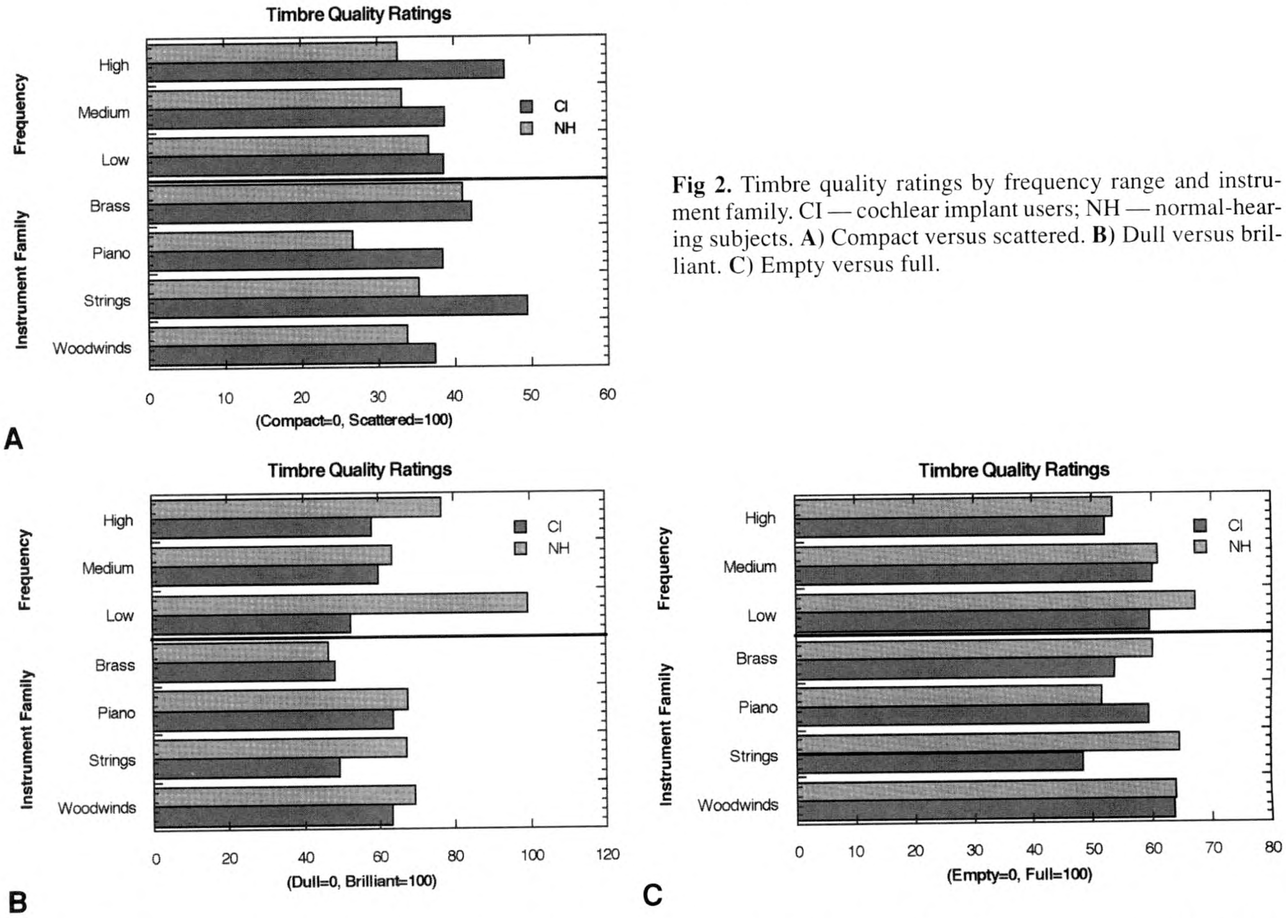

Fig 2. Timbre quality ratings by frequency range and instrument family. CI — cochlear implant users; NH — normal-hearing subjects. **A**) Compact versus scattered. **B**) Dull versus brilliant. **C**) Empty versus full.

cipients, the lower the frequency within each family, the more positive the ratings. For normal-hearing listeners, a difference in frequency range resulted in a significant difference in appraisal for only the string family.

There were no significant differences among the users of the 4 implant devices in general appraisal of timbre. The mean (±SD) appraisal ratings were as follows: Clarion, 49.01 ± 19.58; Ineraid, 44.69 ± 6.08; Med-El, 51.78 ± 15.09; and Nucleus CI22M, 52.51 ± 7.67. The correlations between appraisal scores and speech scores were weak (Iowa Consonant, r = .07 in noise, r = .14 without noise; Iowa Vowel, r = .16; Sentence Test, r = .14; NU-6 Word Recognition, r = .17). However, there were significant correlations between general appraisal ratings and cognitive measures (VMT1, r = .48, p < .001; VMT2, r = .45, p < .003; SCT, r = .48, p < .0009).

Timbre Appraisal Test B: Qualitative Ratings for Three Dimensions of Timbre. Twenty-four normal-hearing adults (6 male, 18 female) completed the qualitative ratings for timbre. They ranged in age from 18 to 72 years (mean, 30.61 years). Fifty-nine adult implant users (24 male, 35 female) completed the qualitative ratings for timbre. They ranged in age from 24 to 81 years (mean, 57.78 years), and had a mean duration of 7 years of implant use (range, 1.8 to 13.56 years). They used one of the following devices: Clarion (N = 20); Ineraid (N = 3); Med-El (N = 10); or Nucleus (SPEAK strategy; CI22M, N = 9; CI24M, N = 17). Thirty-nine patients in this sample also took the Timbre Recognition test, and 42 also took Timbre Appraisal Test A.

An analysis of variance revealed significant interactions for the groups (normals versus implant users) by frequency range (high, medium, low) and the groups by instrumental family (brass, woodwind, piano, strings). We conducted *t*-tests to determine the source of differences.

Differences Between Groups by Frequency. Figure 2 shows the mean differences examined with *t*-tests to compare the 2 groups by frequency for each of the bipolar adjectives (compact at 0, scattered at 100; dull at 0, brilliant at 100; and empty at 0, full at 100). Whereas a larger score for the adjectives "brilliant" and "full" indicates better sound quality, a higher score for the adjective "scattered" indicates more noise and a generally less desirable sound quality. Of particular note, implant recipients found that as a group, those instruments played in the highest frequency range sounded significantly more scattered (ie, noisy; p < .003) and less brilliant (ie, more dull;

TABLE 2. TIMBRE APPRAISAL TEST B: QUALITATIVE RATINGS OF THREE DIMENSIONS OF TIMBRE

	Brilliant = 100; Dull = 0		*Full = 100; Empty = 0*		*Scattered = 100; Compact = 0*	
	NH	CI	NH	CI	NH	CI
Brass						
Trumpet (high)	63.96 ± 19.47	53.63^{a} ± 19.47	64.56 ± 16.23	53.46^{b} ± 20.81	37.98 ± 20.14	44.54 ± 18.85
Trombone (low)	28.96 ± 10.57	42.91^{c} ± 25.02	55.21 ± 21.75	53.99 ± 23.99	44.17 ± 19.32	40.14 ± 19.82
Woodwind						
Flute (high)	80.31 ± 14.99	62.75^{d} ± 20.59	62.38 ± 23.74	61.19 ± 21.75	34.27 ± 24.85	39.03 ± 19.98
Clarinet (medium)	63.62 ± 21.64	61.66 ± 18.18	60.31 ± 21.03	63.20 ± 16.42	34.00 ± 18.78	36.68 ± 17.36
Saxophone (low)	63.38 ± 18.72	65.43 ± 16.82	69.42 ± 14.25	66.84 ± 16.36	32.88 ± 20.15	36.41 ± 19.13
Piano						
High	72.46 ± 19.54	62.73 ± 19.51	45.08 ± 22.31	55.75^{e} ± 22.05	25.79 ± 18.58	41.25^{f} ± 21.14
Low	62.63 ± 26.42	64.77 ± 20.08	58.10 ± 22.72	63.44 ± 17.18	27.71 ± 17.81	35.39 ± 20.37
String						
Violin (high)	77.75 ± 15.85	49.48^{d} ± 28.00	52.50 ± 24.80	39.02^{a} ± 23.88	37.77 ± 26.67	59.63^{g} ± 22.42
Cello (low)	56.19 ± 25.58	49.30 ± 22.15	76.85 ± 18.86	51.65^{d} ± 19.47	32.94 ± 24.44	39.45 ± 19.88

Data are mean ± SD.

NH — normal-hearing subjects; CI — cochlear implant users.

$^{a}p < .03$; $^{b}p < .01$; $^{c}p < .0006$; $^{d}p < .0001$; $^{e}p < .04$; $^{f}p < .003$; $^{g}p < .001$.

$p < .0001$) than did normal-hearing listeners.

Differences Between Groups by Instrumental Family. The *t*-tests revealed significant differences between the 2 groups for 2 instrumental families: piano and strings. In particular, implant recipients rated the string family as less desirable in tone quality on all 3 dimensions (more scattered, $p < .0014$; less full, $p < .0001$; and more dull, $p < .0001$; Fig 2). Table 2 shows the mean ratings by group on these bipolar adjective ratings for individual instruments.

DISCUSSION

This study is consistent with prior studies with smaller sample sizes indicating that implant recipients are significantly less accurate than normal-hearing adults in recognizing musical instruments by their sound. Thus, implant recipients can recognize instruments by sound only to some extent. With respect to timbre recognition among implant users, the Nucleus (CI22; multipeak [MPEAK] and SPEAK) users were less accurate than users of the Clarion, Ineraid, and Med-El processors. Because the Nucleus CI22 uses feature extraction strategies, that particular device would transmit a limited portion of the spectral features, possibly making timbre recognition more difficult. These findings may not reflect the capability of users of more recent devices (eg, Nucleus CI24M) or strategies (eg, continuous interleaved sampling [CIS], simultaneous analog stimulation [SAS], or advanced combination encoder [ACE] processing strategies).

It is interesting to note that 1 subject, who is a professional musician (Med-El processor, Ineraid implant) and who achieves strong speech recognition scores, correctly identified all items in the timbre recognition task. Since implantation, she has devoted considerable time and energy to "relearning" the sounds of the various instruments, and she is once again employed as a music teacher. Her performance on this test even exceeded that of several normal-hearing listeners who claimed prior knowledge of all of the instruments. These results, along with prior findings regarding training by Fujita and Ito,[7] are promising with regard to potential implant benefit as assessed with this particular music listening task. Evidently, the device, while imperfect, does provide enough of the spectral features to permit recognition of various timbral qualities.

With regard to qualitative descriptions of instruments, it is interesting to note the lack of significant differences among these 4 devices. Evidently, none of these signal processors are clearly superior or inferior with regard to naturalness or richness of sound, despite important differences in the hardware and software. As is the case for many speech recognition measures, individual user differences appear to have a greater impact on test outcomes than do differences in these particular devices. These data also support previous studies that show speech recognition to be very weakly correlated with timbre appraisal, whereas cognitive measures of sequential processing predict both music perception and speech perception.[2,4]

These data emphasize the interaction of particular structural features of musical stimuli with the peripheral hearing mechanism with regard to response to timbral quality. Whereas prior research indicates that normal-hearing adults tend to rate musical sounds with more high-frequency energy with the verbal descriptor "brilliant,"[14,20] these implant recipients rated those instruments played in the higher frequency range as significantly more scattered (ie, noisy) and dull in tone quality than did the normal-hearing sample. Perhaps the emphasis on the high-frequency range in some implant maps results in a disproportionate amplitude of overtones higher than those that would be heard by a normal ear. This effect may result in a distorted, harsh, or shrill sound, rather than the more positive "brilliant" descriptor that normal-hearing people typically associate with high-frequency instruments. The poor quality in the higher frequencies may also be related to poorer nerve survival in the basal region of the cochlea, which is stimulated by the implant in response to higher-frequency sounds.[21] The harsher or scattered quality of the higher frequencies is consistent with clinical observation by our audiologists in speech perception tasks that implant recipients often find the sound quality delivered by the 2 most basal electrodes to be unpleasant in quality.

With regard to everyday music listening satisfaction, the key finding of this study is that users of current-day implants, although able to distinguish between or identify some instruments by name, are less accurate at instrumental recognition than normal-hearing listeners. Furthermore, cochlear implant recipients do not find the sound of many musical instruments as pleasant as do normal-hearing listeners (especially for higher-frequency sounds), nor do the instruments retain the characteristic features of brilliance, clarity, and richness so important with regard to the uniqueness and beauty of individual instrumental sounds. Thus, it appears that changes in signal processing targeted toward music listening, in contrast to the usual speech coding strategies, may be necessary in order for more implant recipients to recognize instruments and perceive a pleasing, natural, and aesthetic sound quality in the wide range of frequencies and spectral variations found in music.

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INCIDENCE OF REGENERATION OF THE CHORDA TYMPANI NERVE AFTER MIDDLE EAR SURGERY

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We retrospectively reviewed 52 patients who underwent middle ear surgery during which the chorda tympani nerves were severed and who then underwent secondary surgery 1 to 5 years later. In 22 patients (42.3%), regenerated chorda tympani nerves (entire length of the tympanic segment) were detected in the submucosal layer of the reconstructed eardrum during the secondary surgery. Before the secondary surgery, 16 patients (30.8%) showed threshold recovery on electrogustometry. When 5 regenerated nerves were observed by transmission electron microscopy, myelinated nerve fibers were detected in a small fascicle or connective tissue, but the number of myelinated axons was significantly decreased (7.4% to 84.6%; $p = .01$) compared with that in normal subjects ($1,911 \pm 324$; $n = 4$). There was a significant difference in the incidence of regeneration between the group with end-to-end anastomosis (5/5 or 100%) and that with nerve gap defects (17/47 or 36.2%; $p < .05$); this finding suggests that repair of the sectioned nerve produces a better incidence of regeneration than leaving the nerve unrepaired.

KEY WORDS — chorda tympani nerve, electrogustometry, filter-paper disc method, nerve repair, regeneration, transmission electron microscopy.

INTRODUCTION

The chorda tympani nerve arises from the mastoid portion of the facial nerve. It enters the middle ear cavity by the posterior canaliculus (iter chordae posterius) and leaves via the anterior canaliculus (iter chordae anterius) in the petrotympanic fissure of Glaser.[1] The chorda tympani is the only nerve in the body that crosses an air cavity (tympanic cavity). Sensory afferent axons of the chorda tympani, most of which might be myelinated fibers,[2] are distributed to the taste end organs (fungiform papillae) on the anterior two thirds of the tongue on one side.

During middle ear surgery, the chorda tympani nerve is frequently severed, with the result of subjective taste disorder.[2-6] In the early years of stapes surgery for otosclerosis, one was frequently advised to "obtain good hearing and patients will be unaware or unconcerned about the taste disturbance."[3(p392)] This concept arose from the fact that no one knew whether the chorda tympani actually regenerated in the tympanic cavity. To date, it has not been clarified how this nerve regenerates after being severed. One reason why there have not been any reports concerning the regeneration of the chorda tympani in humans is the difficulty in identifying regenerated nerves. The only way to identify a regeneration of the chorda tympani nerve after the nerve has been severed is by secondary surgery. In 1992, we found the first patient who showed with regeneration of the chorda tympani nerve during subsequent surgery (unpublished observations), and thereafter, we paid strict attention to regenerated nerves. The first report of 3 clinical patients with regenerated chorda tympani nerves was published elsewhere.[7]

The present study evaluated the incidence of chorda tympani regeneration detected during secondary surgery performed after the nerve was severed. Furthermore, it also evaluated whether repair of the sectioned chorda tympani nerve produces a better incidence of regeneration than leaving the nerve unrepaired. In this study, these questions related to chorda tympani regeneration were discussed on the basis of surgical findings of secondary surgery and morphological findings of regenerated nerves on transmission electron microscopy. Functional analysis using the filter-paper disc method and electrogustometry (EGM) was also conducted.

MATERIALS AND METHODS

Subjects. Fifty-two patients in whom the chorda tympani was cut during middle ear surgery and who underwent staged tympanoplasty or reoperation were evaluated. Of the 52 patients, 50 underwent the second-stage surgery of staged tympanoplasty 11 to 19 months after the primary surgery, and the other 2 underwent reoperation 47 to 65 months after the initial

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surgery. The patients' ages ranged from 3 to 77 years, and the group included 32 male patients and 20 female patients. The underlying disease was chronic otitis media with cholesteatoma in all cases.

The chorda tympani nerves were severed during the initial surgery to eradicate cholesteatoma, and a nerve gap was created between the proximal and distal stumps. In 5 patients, nerve repair (end-to-end anastomosis) was possible, and proximal and distal stumps were anastomosed without suture on the temporal muscle fascia by the underlay method used to reconstruct the eardrum. Even if a nerve gap was present, the proximal and distal ends were placed on the temporal muscle fascia almost in the original position without a nerve graft and fixed with fibrin glue (Beriplast, Hoechst Marion Roussel, Tokyo, Japan). Depending on the gap between the proximal and distal ends fixed on the temporal muscle fascia, 4 classifications of patients were made: 1) end-to-end anastomosis, 2) 1- to 3-mm nerve gap, 3) 4- to 6-mm nerve gap, and 4) nerve gap of more than 7 mm.

During secondary surgery, strict attention was paid to whether the chorda tympani nerves had regenerated. From the number of patients in whom the entire length of the tympanic segment of the chorda tympani nerve was detected, the incidence of regeneration of the nerve was determined.

Morphological Study of Regenerated Chorda Tympani. Surgical findings of regenerated chorda tympani nerves detected during secondary surgery were evaluated. In 5 patients, morphological observation of the regenerated chorda tympani was performed by transmission electron microscopy. A 2- to 3-mm distal segment was removed from the cut end of the regenerated chorda tympani. These nerves had to be re-severed to eradicate residual or recurrent cholesteatoma. The resected segment of the nerves was fixed by immersion in a fixative solution (2% paraformaldehyde and 0.5% glutaraldehyde in 0.1 mol/L phosphate-buffered saline [PBS; pH 7.4]) for more than 2 days, embedded in gelatin (20% in PBS), and submerged in a 20% sucrose solution in PBS (pH 7.4) at 4°C for 24 hours. Transverse frozen sections of 65-μm thickness were cut with a cryostat and postfixed for 2 hours with 1% osmium tetroxide at 4°C, dehydrated with a series of graded alcohols, and embedded in Epon 812. Ultrathin sections were cut and mounted on a Formvar-coated keyhole grid and observed with a transmission electron microscope (Hitachi H-7000) at a magnification ranging from ×1,000 to ×2,000 of the entire cross-sectional area. Myelinated fibers were counted on photographic montages.[7] Histograms of fibers were also made from the montages. Since very few unmyelinated fibers could be detected, probably because of problems encountered during the fixation and staining process, they were not counted in this study.

As controls, chorda tympani nerves were taken from 4 patients who had chronic otitis media with cholesteatoma and showed a normal threshold on EGM. These nerves had to be severed to eradicate the cholesteatoma. The resected nerves were observed by transmission electron microscopy.

Electrogustometry. Functional observation by EGM was performed before and after the operation. The electrical stimulus was delivered via an electrogustometer (EG-IIB, Nagashima Medical Instruments Co, Tokyo) with a single, flat, circular stimulus probe (5 mm in diameter) made of stainless steel. The stimulus rod was placed on the tongue with the stimulus probe resting 2 cm laterally from the anterior midline and 2 cm from the tip of the tongue. This area is the so-called intrinsic region dominated by the chorda tympani nerve. An ascending threshold procedure was used. A 500-ms anodal stimulation was presented unilaterally, beginning at −8 dB and proceeding to 34 dB (3 to 400 μA) in 2-dB steps as described in a previous report.[8] Differences in thresholds between the surgical and nonsurgical sides were recorded. The threshold difference between the two sides of the tongue in normal subjects was within 4 dB, as reported elsewhere.[9]

Filter-Paper Disc Method. After the secondary surgery, the perception of taste was evaluated by the filter-paper disc method. A commercial assay kit (Taste Disk, Sanwa Chemicals, Nagoya, Japan) was used.[9] The taste solutions consisted of sucrose, sodium chloride, tartaric acid, and quinine hydrochloride at 5 concentration gradations (concentrations 1 to 5) for each solution. A 5-mm filter-paper disc was dipped into a test solution and placed on the test site on the tongue, as in EGM. The taste threshold was obtained by testing from the lowest concentration. The number for the lowest concentration at which the taste was perceived was recorded as the threshold value. When a response was not observed at the highest concentration (5), no response was entered.

Statistical Analysis. Data from each group were compared by the standard χ^2 test and Fisher's exact test. A p value of less than .05 was considered significant.

RESULTS

Surgical Findings of Regenerated Chorda Tympani. In 22 patients, regenerated nerves were detected in the submucosal connective tissue layer of the reconstructed eardrum, and were easily exposed by a fine needle (Fig 1). The course of the regenerated nerves in the tympanic segment was similar to that of nor-

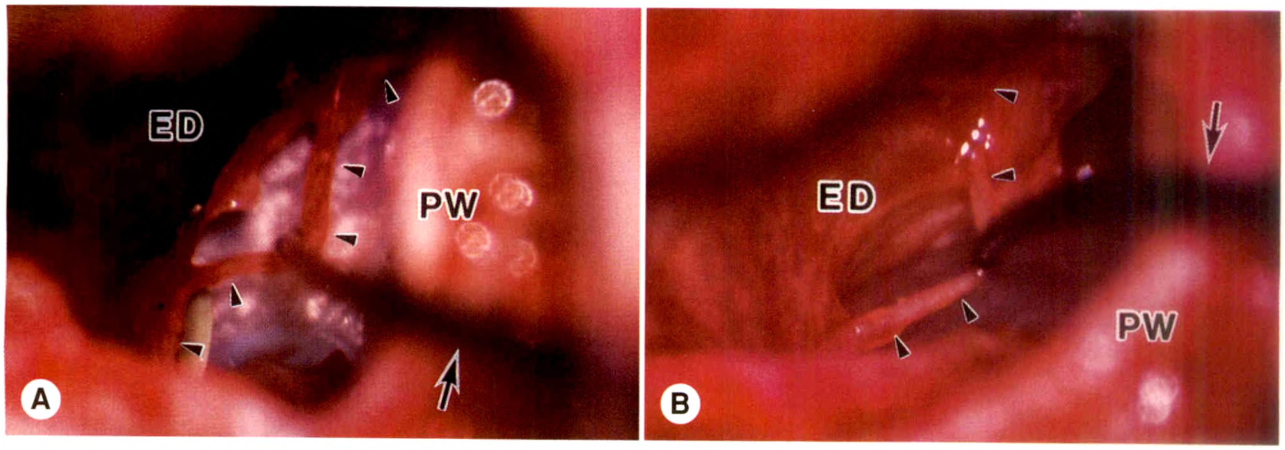

Fig 1. Intraoperative findings of regenerated chorda tympani nerves. Regenerated nerves existed in submucosal connective tissue layer of reconstructed eardrum. All nerves of tympanic segment were exposed and stretched with fine needle. ED — mucosal surface of reconstructed eardrum; PW — posterior wall of external auditory canal; arrowheads — exposed chorda tympani nerves; arrow — needle. **A**) Case 6. **B**) Case 8.

mal nerves, except that the nerve did not run through the aerated tympanic cavity, but inside the reconstructed eardrum. In 11 of the 22 patients, the regenerated chorda tympani was touched when the nerve was exposed during the secondary surgery. In the other 11 cases, the regenerated nerves were cut again to eradicate residual or recurrent cholesteatoma.

Incidence of Regeneration of Chorda Tympani. Since regenerated nerves were found during the secondary surgery in 22 of the 52 patients, the incidence of regeneration was established as 42.3%. In 9 of the 13 pediatric patients under 10 years old (66.7%), regenerated chorda tympani nerves were detected. There was a significant difference between patients under 10 years old and those over 40 (5/18 or 27.8%; $p < .05$).

Relationship Between Length of Nerve Gap Defects and Regeneration. Table 1 shows the relationship between the length of the nerve gap defects and the incidence of nerve regeneration. In all patients with direct end-to-end anastomosis ($n = 5$), regenerated chorda tympani nerves were detected. There was a significant difference between the group with end-to-end anastomosis ($n = 5$) and that with a nerve gap defect ($n = 47$; $p < .01$) and also between the group with regenerated nerves ($n = 22$) and that with no regeneration ($n = 30$; $p < .01$). Even when there was a total or subtotal defect in the tympanic segment of the chorda tympani (at least 7 mm), the nerve was able to regenerate in 21% of patients.

TABLE 1. RELATIONSHIP BETWEEN LENGTH OF NERVE GAP DEFECT AND REGENERATION OF CHORDA TYMPANI NERVES IN PATIENTS WHO UNDERWENT SECONDARY SURGERY (N = 52)

	No Regeneration of Nerve (n = 30)		*Regeneration of Nerve* (n = 22)*	
Nerve Gap	*No.*	*%*	*No.*	*%*
End-to-end anastomosis (n = 5)		0	5	100†
1 to 3 mm (n = 7)	3	43	4	57
4 to 6 mm (n = 11)	4	36	7	64
≥7 mm (n = 29)	23	79	6	21

*$p < .01$ compared to group with no regeneration of nerve ($n = 30$).
†$p < .01$ compared to group with nerve gap ($n = 47$).

Morphological Findings of Regenerated Chorda Tympani. In 5 regenerated nerves, myelinated nerve fibers regenerated in a small fascicle or connective tissue, but the number of myelinated fibers was decreased and the distribution was sparse (Fig 2). The total number of regenerated myelinated fibers ranged from 141 to 1,616 (mean ± SD, 788 ± 590). Since the total number of myelinated fibers was 1,911 ± 324 in the normal subjects ($n = 4$), the number of regenerated nerve fibers varied from 7.4% to 84.6% of that in normal subjects (Table 2[7]). The diameter of the regenerated myelinated fibers was smaller than that of normal fibers (Fig 3).

Functional Results Measured by EGM and Filter-Paper Disc Method. The EGM thresholds measured within 1 month after severing of the chorda tympani nerves showed no response in 19 of the 22 patients with regenerated nerves. In 3 other pediatric patients, EGM could not be performed before and shortly after the nerve was severed. Subjective complaints of taste disorder after severing of the nerve were made by 7 of the 22 patients (31.8%), but the complaints disappeared within 6 months to 1 year after primary surgery in all cases.

Before the secondary surgery, 16 (16/52 or 30.8%) of the 22 patients, including 4 of 5 patients with regenerated fibers detected by transmission electron microscopy, showed threshold recovery on EGM, whereas the other 6 patients showed no response. This out-

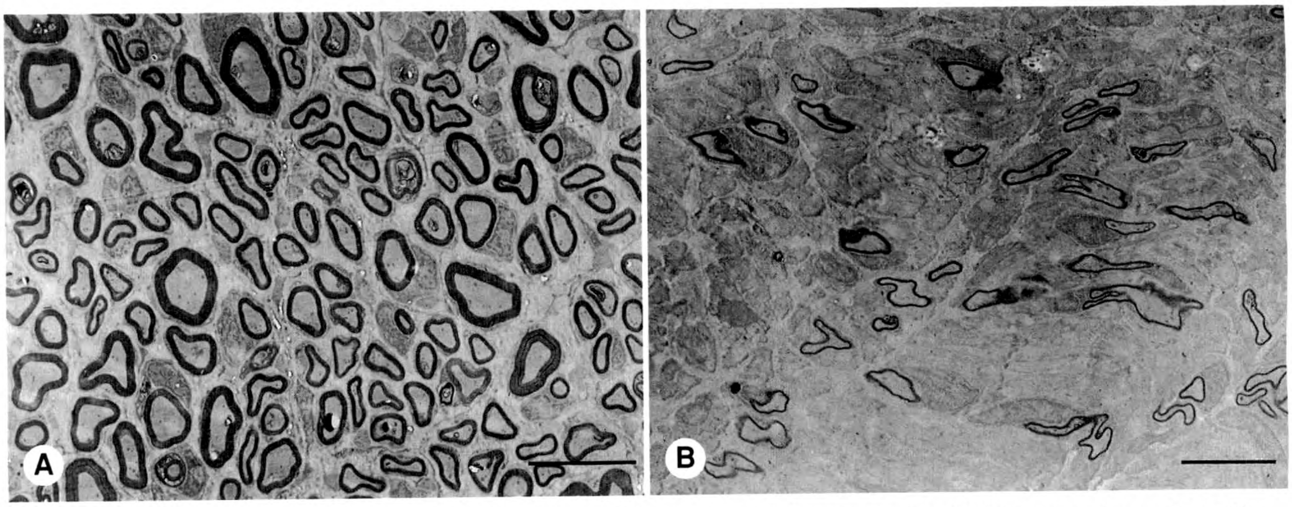

Fig 2. Transmission electron microscopic findings. Bar — 10 µm. **A)** Normal nerve (case 4). **B)** Regenerated nerve (case 8).

come suggests that an interval of around 1 year between the primary and secondary operations might be insufficient for regenerating axons to reach the taste end organs. In these 16 patients with EGM recovery, the EGM thresholds showed complete recovery (±0 dB) in 1 pediatric patient and incomplete recovery (+6 to +28 dB) in 15 patients as compared with the nonsurgical side. Since the regenerated chorda tympani nerves in 11 of the 22 patients were cut again during the secondary surgery, only 8 patients showed a response to electric stimulation when measured by EGM after the secondary surgery. In these 8 patients, taste was perceived in all or some of the sweetness, saltiness, sourness, and bitterness categories when measured by the filter-paper disc method. The fact that the threshold values on the surgical side were higher than those on the nonsurgical side suggests that recovery of taste was incomplete in these patients with regenerated chorda tympani nerves.

DISCUSSION

In this study, the incidence of chorda tympani nerve regeneration was 42.3%. Since nerve regeneration was detected by macroscopic inspection during secondary surgery, a morphological study of the regenerated nerves was performed. As a result, myelinated nerve fibers were detected in all 5 samples of the regenerated nerves by transmission electron microscopy. However, it remained obscure whether the regenerated axons reached the taste end organs, because regeneration of the nerve was detected only in the tympanic segment. To answer this question, functional analysis was essential. As gustatory function tests, EGM and a filter-paper disc assay using taste solutions were selected for quantitative evaluation of the taste threshold in each patient. As a result, recovered gustatory function was verified in 16 of the 22 patients with regenerated nerves — a finding suggesting that 72.7% of the regenerated nerves had actual taste function.

Certain conditions are considered contributory to regeneration of the chorda tympani. One is the age of the patient. In this study, there was a significant difference between patients under 10 years old and those over 40 ($p < .05$). Other factors are the direction of the remaining parts of the nerve and the dis-

TABLE 2. NUMBER OF MYELINATED FIBERS AND MAXIMAL DIAMETER OF NORMAL AND REGENERATED CHORDA TYMPANI NERVES

Cases With Normal Nerves	*Age (y)*	*No. of Myelinated Fibers*	*Diameter of Nerves (µm)*	*Cases With Regenerated Nerves*	*Age (y)*	*Nerve Gap**	*Regenerated Fibers*		*Diameter of Nerves (µm)*
							No.	*%†*	
Case 1§	6	1,693	379	Case 5§	48	End-to-end	903	47.3	243
Case 2§	51	1,840	500	Case 6§	29	End-to-end	979	51.2	289
Case 3§	54	1,723	242	Case 1§	7	6 mm	141	7.4	387
Case 4	16	2,388	429	Case 7	8	End-to-end	1,616	84.6	379
				Case 8	9	4 mm	301	15.8	339
Mean ± SD		1,911 ± 324 (n = 4)	388 ± 109	Mean ± SD			788 ± 590 (n = 5)		327 ± 61

*Distance between proximal and distal cut ends after nerve was severed during primary surgery (or end-to-end anastomosis).

†Percentage of regenerated nerve fibers compared with mean value (1,911) of normal nerve fibers.

§Previously reported elsewhere.[7]

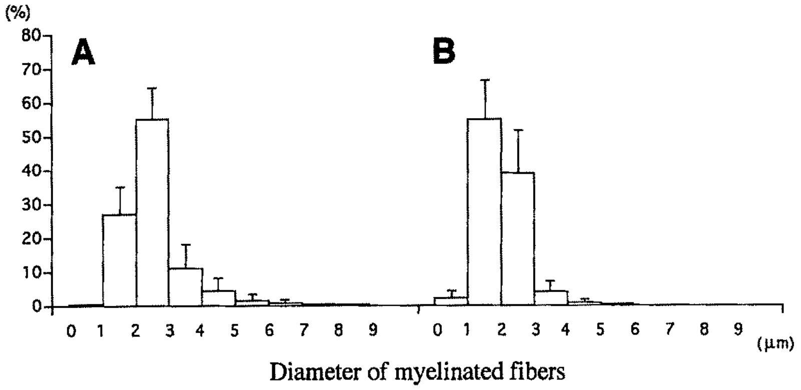

Fig 3. Histograms of myelinated nerve fibers. **A)** Normal nerves (n = 4). **B)** Regenerated nerves (n = 5).

tance between the central and peripheral ends. If the nerve is severed and a nerve gap is created, it is usually difficult to anastomose the proximal and distal stumps of the nerve, because the chorda tympani runs across an aerated cavity. Degenerative changes that occur after peripheral nerves are severed may compromise the level of recovery of the regenerating axons, and it is commonly assumed that nerve repair will result in a better level of recovery.[10] In this context, some authors clearly indicate better recovery after repair of animal sensory nerves such as the sciatic, peroneal, and cutaneous nerves.[11-15] Although direct end-to-end anastomoses were possible in 5 of the 22 patients demonstrating regenerated nerves in this study, gap separation remained in the other 17 patients, and there was a significant correlation between the length of the nerve gap and regeneration of the nerve ($p < .01$). These results suggest that nerve repair is very important, although regeneration of the nerve is possible even if there is a gap between the central and peripheral cut ends. In addition, the use of temporal muscle fascia seems to have played an important role in regenerating the chorda tympani nerve, because all regenerated chorda tympani nerves were detected in the submucosal connective tissue layer of the reconstructed eardrum.

It has been reported that fibrin glue has an inhibitory effect on neural regeneration, resulting in inferior results compared with conventional suture repair.[16] In this study, the proximal and distal parts of the severed nerves were fixed with fibrin glue on the temporal muscle fascia, and some of the cut ends may have been coated with fibrin glue. Nevertheless, regeneration could be detected in 17 (36.2%) of the 47 severed nerves with nerve gap defects (Table 1) — a finding suggesting that fibrin glue did not completely inhibit neural regeneration. However, it is supposed that the incidence of regeneration of the nerve fibers would have been increased if fibrin glue had not been used to fix the cut nerve ends. A trial will be performed in our clinic.

There have been numerous animal studies on regeneration of the chorda tympani or lingual nerve,[17-23] but there have been few studies in humans after severing of the nerve.[5-7] In the animal studies using hamsters, chorda tympani nerves were cut or crushed in the middle ear with the result of regeneration of the nerve to the tongue.[21,22] On morphological observation of regenerated chorda tympani nerves 4 months after the nerves had been cut, nerve regeneration was found near the tongue, but there were 67% fewer myelinated fibers than there were in controls on transmission electron microscopic observation.[22] These fibers were sufficient to record the recovery of electrophysiological taste responses and to support normal taste bud sizes and numbers. In the present study, the total number of myelinated axons in regenerated chorda tympani nerves was 7.4% to 84.6% of that of normal chorda tympani nerves ($p = .01$). These findings were in good agreement with those of a hamster study.[22] A study of a human temporal bone collection demonstrated regenerated myelinated fibers in the anterior canaliculus in 6 of 9 patients in whom the chorda tympani nerves had been cut during middle ear surgery.[24] This study, however, did not provide evidence that the regenerated nerve fibers ended at the taste end organs, because of the lack of functional data. Our results demonstrated regeneration of the nerve in humans and recovered gustatory function in some cases.

Although myelinated nerve fibers were observed in the small fascicle covered with connective tissue by observation of the entire section of the regenerated chorda tympani nerves on transmission electron microscopy, nerve fibers may regenerate by various circuitous routes. Regenerated nerve fibers may be transmitted through various mucosal surfaces underneath the tympanic membrane graft or through the fibrous tissue within the tympanic graft. To clarify the route of regeneration, evaluation of human temporal bones with regenerated chorda tympani nerves will be needed.

Unmyelinated fibers were not counted, because of the technical problems involved in the present study.

On the basis of a previous report that afferent fibers accounted for 79% of all myelinated, but only 30% of all unmyelinated, fibers in the rat chorda tympani nerve,[25] Ylikoski et al[2] suggested that it would appear that the efferent function (secretory) was represented mainly by unmyelinated nerve fibers and that most of the afferent fibers (sensory function) are myelinated in humans, if the human chorda tympani fibers have a relationship similar to that in the rat. Thus, it is considered that the myelinated fibers distributed to the anterior two thirds of the tongue were mainly afferent gustatory fibers. Although it remains obscure whether the regenerated fibers should be classified as efferent or afferent, even after morphological analyses, afferent fibers are thought to be mainly represented among the regenerated myelinated fibers in our series because incomplete recovery of electrogustation was observed in patients with regenerated chorda tympani nerves, while electrogustation did not recover in patients with nonregenerated nerves.

It has been reported that 44% to 80% of patients whose chorda tympani was severed during middle ear surgery complained of taste disorder.[3,4] Taste disorder in the majority of patients, however, improved within 1 to 2 years. In our study, the time course of subjective improvement in taste showed a similar tendency. Taste disorder was a complaint in 32% of the patients, but this complaint improved within 1 year in all patients. One of the possible reasons for an unchanged whole-mouth taste sensation after surgery is the compensation mechanism (release of inhibition by the chorda tympani) of the glossopharyngeal nerve, which provides sensory innervation to the pharynx and base of the tongue.[26,27] However, the compensation mechanism could not be observed by EGM in any of the patients in this study. Therefore, the problem of unchanged whole-mouth taste sensation after surgery cannot be explained solely by this mechanism. Further study will be needed to clarify the mechanism of unchanged whole-mouth taste function after the chorda tympani nerve is severed.

CONCLUSION

Chorda tympani nerves were regenerated in 22 of 52 patients (42.3%) in whom the nerves were severed during middle ear surgery. The regenerated nerves were found in the submucosal connective tissue layer of the reconstructed eardrum during secondary surgery. The incidence of regeneration of the nerve was 100% in 5 patients with end-to-end anastomosis, whereas it was 36% in patients with nerve gap defects; this was a significant difference. These results indicate that nerve repair might be desirable to promote regeneration of the chorda tympani nerves. Further examination of gustatory function in patients with regenerated chorda tympani nerves will be necessary to elucidate whether all regenerated nerves actually have taste function.

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CARTILAGE INTERPOSITION IN OSSICULOPLASTY WITH HYDROXYLAPATITE PROSTHESES: A HISTOPATHOLOGIC STUDY IN THE GUINEA PIG

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In this experimental animal study, a cartilage disk was interposed between a synthetic middle ear prosthesis and the tympanic membrane in guinea pigs to investigate its effect on the extrusion process of the implant. Two groups of guinea pigs were studied. One group consisted of animals in which the prosthesis was directly in contact with the tympanic membrane, and the other group consisted of animals in which a cartilage disk had been inserted between the head of the prosthesis and the tympanic membrane. Before histologic processing, in situ inspection was performed with an operating microscope. After fixation and embedding, light microscopic and transmission electron microscopic examination were performed. We studied the histopathologic aspects of the tympanic membrane with regard to the protrusion and extrusion processes of the middle ear implant. In this experimental model, protrusion and extrusion of a hydroxylapatite middle ear prosthesis was greatly reduced by interposition of a cartilage disk. Further clinical evaluation of these experimental results is needed in the human middle ear.

KEY WORDS — cartilage interposition, hydroxylapatite, ossiculoplasty.

INTRODUCTION

In tympanoplasty, otologic surgeons have successfully used biocompatible synthetic materials to reconstruct the middle ear air conduction system. Synthetic implants are greatly preferred to autografts and allografts.[1] At the moment, hydroxylapatite is the most used synthetic material for reconstruction of the ossicular middle ear chain in tympanoplasty. Hydroxylapatite is a calcium phosphate ceramic that resembles the mineral matrix of living bone tissue. It is rapidly covered by epithelial cells and permits differentiation into goblet and ciliated cells without any foreign body reaction. Hydroxylapatite is completely bioactive, biocompatible, and osteoconductive.[2-5]

There used to be intrinsic problems of rejection and extrusion for all synthetic implants. However, with the introduction of highly biocompatible materials, rejection is seldom observed anymore. Extrusion of a biocompatible ceramic middle ear implant in direct contact with the tympanic membrane is probably caused, not by a foreign body reaction, but by pressure necrosis of the membrane.[6-10] Extrusion rates can be lowered by interposing a small cartilage disk between a synthetic implant and the tympanic membrane.[1,6-15] Cartilage has the advantage of being well tolerated by the tympanic membrane. It can be placed under some tension, and has a very low risk of extrusion. The literature refers to average extrusion rates of biocompatible implants as varying from 15% to 22%; interposing a cartilage disk reduces extrusion rates to 3% to 6%, which are comparable to those of transplants.[1,6-12]

In this study, we implanted hydroxylapatite middle ear prostheses in the bullae of guinea pigs. The guinea pig temporal bone and its contents are similar to those of humans, but there are some minor differences. In regard to the temporal bone dimensions, the dimensions of the eardrum and the tympanic ring are greater in the guinea pig than in humans, and the tympanic membrane in the guinea pig is devoid of the pars flaccida.[16]

Two groups of guinea pigs were investigated: the first group consisted of cases in which a hydroxylapatite prosthesis was directly in contact with the tympanic membrane, and the second group consisted of cases in which a cartilage slice had been inserted between the head of the hydroxylapatite prosthesis and the tympanic membrane. The purpose of this study was to evaluate the histopathologic aspects of the tympanic membrane and the differences in extrusion and protrusion rates between the groups of guinea pigs.

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MATERIALS AND METHODS

In this study, middle ear surgery was carried out on 21 female pigmented guinea pigs weighing about 450 g. The methods of animal care and use were approved by the Experimental Animal Committee of the University of Groningen, protocol number 1174, in accordance with the principles of the Declaration of Helsinki.

OPERATION TECHNIQUE

The animals were anesthetized by an intramuscular injection of Ketalar-Rompun (0.7/0.3). All operations were performed by the same surgeon using a Zeiss stereo microscope. The body temperature was maintained with an electric heating pad.

Tympanotomy was performed by the inferior approach.[17,18] After the perichondrium covering was stripped off, the bulla was opened by creating a square shutter (4 × 4 mm). The prosthesis was custom-made for this experiment. The head of the prosthesis consisted of hydroxylapatite (diameter, 2.0 mm), and the shaft was made of a spiral spring that was attached to the prosthesis head with dental glue. The prosthesis was implanted between the tympanic membrane and the bulla wall under minimal tension. The shaft had no contact with middle ear structures other than the bulla wall at its end. In half of the cases, a slice of cartilage with a thickness of 0.3 to 0.5 mm was interposed between the prosthesis head and the tympanic membrane, with the perichondrium covering opposed to the tympanic membrane. The prostheses including the cartilage disk had approximately the same length as the length of the prostheses without cartilage interposition. The cartilage disk, with the overlying perichondrium, was obtained from the auricle. After adequate positioning of the prosthesis, the square shutter was replaced on the bullar defect and fixed with Histoacryl Tissuecol, and wound closure was performed. After 6 months, the guinea pigs were painlessly sacrificed by decapitation.

HISTOLOGIC PROCESSING

After decapitation, the bullae were opened and fixed in 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4. The specimens were then decalcified for 5 days in 10% ethylenediaminetetraacetic acid, pH 7.4, postfixed in 1% osmium tetroxide with 1% $K_4Ru(CN)_6$ for 2 to 3 hours, carefully rinsed in distilled water, dehydrated in a graded ethanol series followed by propylene oxide, and infiltrated with a mixture of 1:1 propylene oxide and Spurr's low-viscosity resin for 2 hours followed by pure resin infiltration overnight. One specimen was embedded in HPMA (hydroxypropyl methacrylate) and stained for elastin. Polymerization took place at 70°C after desiccation in a vacuum. The specimens were cut in semithin slices (2 µm), and stained with toluidine blue for evaluation by light microscopy (LM). A selection of 6 pieces were further examined by transmission electron microscopy (TEM). Ultrathin sections of 100 nm were contrast-stained with 7% uranyl acetate in 70% methanol and lead citrate. Evaluation was performed with a Philips 201 transmission electron microscope operating at 40 kV.

EXAMINATION

Before histologic processing, in situ inspection of the position, protrusion, and extrusion of the prosthesis was performed with a Zeiss stereo operating microscope.

After fixation and embedding, LM was performed. We studied the histopathologic aspects of the tympanic membrane, including signs of protrusion or extrusion of the middle ear prosthesis. Protrusion was defined as prominence of the prosthesis in the tympanic membrane, with the tympanic membrane still intact. Extrusion was defined as prominence of the prosthesis in the tympanic membrane resulting in discontinuity of the tympanic membrane.

In addition to the LM evaluation, we also performed TEM evaluations in selected cases. Six cases (5 with cartilage interposition, 1 without cartilage interposition) of apparently normal tympanic membranes as observed with LM were further investigated with TEM. Also, 3 left control ears without intervention were studied by TEM.

RESULTS

We initially implanted hydroxylapatite middle ear prostheses in the right bullae of 21 guinea pigs (11 without cartilage interposition, 10 with cartilage interposition). During the 6-month follow-up period, 3 guinea pigs died: 2 guinea pigs in which cartilage was interpositioned, and 1 guinea pig without cartilage interposition. There was no clear cause of death. Also, 2 guinea pigs (with cartilage interposition) were excluded after being prepared for evaluation, for technical reasons: in 1 guinea pig, the prosthesis was situated against the bulla wall and therefore had no contact with the tympanic membrane, and in the second one, the fixation and embedding procedure had failed. Consequently, we studied 16 guinea pigs: group A consisted of 10 guinea pigs in which the prosthesis was directly in contact with the tympanic membrane, and group B consisted of 6 guinea pigs in which a cartilage disk had been inserted between the head of the prosthesis and the tympanic membrane. Besides the 2 implantation groups, we studied the histologic aspects of a group of 6 left control ears that had received no intervention.

TABLE 1. FINDINGS IN TYMPANIC MEMBRANE AFTER IMPLANTATION OF HYDROXYLAPATITE MIDDLE EAR PROSTHESES WITH OR WITHOUT CARTILAGE INTERPOSITION

	In Situ Inspection	*Light Microscopy*
No cartilage (n = 10)	Protrusion or extrusion 7	Extrusion 3
	Questionable protrusion or extrusion 1	Protrusion 7
	Normal 2	
Cartilage (n = 6)	Protrusion or extrusion 1	Extrusion 1
	Normal 5	Protrusion 1
		Normal 4

IN SITU INSPECTION

Of the 16 right bullae, we observed 15 healthy middle ear cavities without any sign of infection or inflammation. Only 1 bulla, in which cartilage was positioned between the prosthesis head and the tympanic membrane, showed purulent otitis media. Of the 16 bullae, all prostheses were in an adequate position and were directly or indirectly in contact with the tympanic membrane; the end of the spiral spring shaft of the prostheses was still in contact with the bulla wall.

We estimated the extrusion and protrusion rates of the prostheses (Table 1): 8 cases of extrusion or protrusion (varying between mild and severe) were observed (7 without cartilage interposition, 1 with cartilage interposition). One case of protrusion or extrusion was questionable (no cartilage interposition) and the resulting 7 cases showed no clear signs of protrusion or extrusion (2 without cartilage interposition, 5 with cartilage interposition). It was impossible to differentiate between extrusion and protrusion.

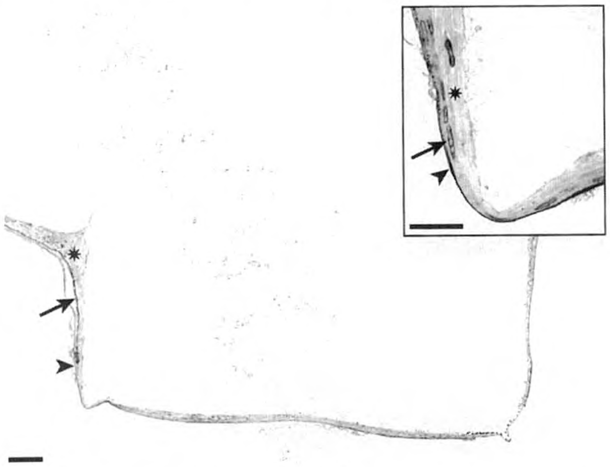

Fig 1. Light microscopy photograph of tympanic membrane after implantation of hydroxylapatite middle ear prosthesis without cartilage interposition. Prosthesis protrudes into tympanic membrane. Middle, fibrous layer (arrow) is atrophic with discontinuity at edge (**inset**); outer, epidermal layer (arrowhead) and inner, mucosal layer (asterisk) are less affected. Bar — 100 µm; inset bar — 20 µm.

TABLE 2. FINDINGS IN TYMPANIC MEMBRANE, WITH EMPHASIS ON MIDDLE, FIBROUS LAYER, AFTER IMPLANTATION OF HYDROXYLAPATITE MIDDLE EAR PROSTHESES WITH OR WITHOUT CARTILAGE INTERPOSITION

	Light Microscopy	*Transmission Electron Microscopy*
No cartilage (n = 10)	Absent 4	
	Atrophic 5	
	Questionable atrophy 1 →	With continuous fibers 1
Cartilage (n = 6)	Absent 1	
	Questionable atrophy 1 →	With continuous fibers 1
	Normal 4 →	With fiber intermissions 3
	→	With continuous fibers 1

LIGHT MICROSCOPY

Control Group. We studied, by LM, 6 tympanic membranes of left control ears that had received no intervention. The tympanic membrane is made up of 3 layers: 1) the outer, epidermal layer, 2) the middle, lamina propria layer (fibrous layer), and 3) the inner, mucous layer, as reported earlier in the literature.[19-21] All of the tympanic membranes had an entirely normal appearance, without any sign of atrophy. The HPMA-embedded tympanic membrane showed elastin in the epidermal and mucosa layers, but not in the fibrous layer.

Group A. In group A (no cartilage interposition, n = 10), all ears except 1 showed severe histopathologic changes of the tympanic membrane (Fig 1). The most prominent changes took place in the middle, fibrous layer. The outer, epidermal layer was less affected. Of all 10 prostheses, 3 prostheses were actually extruded, and the rest protruded (Table 1). The changes in the middle, fibrous layer ranged from mild to severe reductions in thickness (atrophy) to discontinuity of the middle, fibrous tissue layer to total absence of this tissue layer. The middle, fibrous layer of the tympanic membrane was questionably atrophic in 1 case, atrophic in 5 cases, and absent in 4 cases (Table 2).

Group B. In group B (cartilage interposition, n = 6), histopathologic examination showed 1 case of extrusion, in the guinea pig with purulent otitis media. In only 1 guinea pig did we observe protrusion of the prosthesis and a tympanic membrane with questionable atrophy of the middle fibrous layer (Tables 1 and 2). The remaining tympanic membranes were all normal, without any sign of atrophy, protrusion, or extrusion (Fig 2). The interposed cartilage disks showed

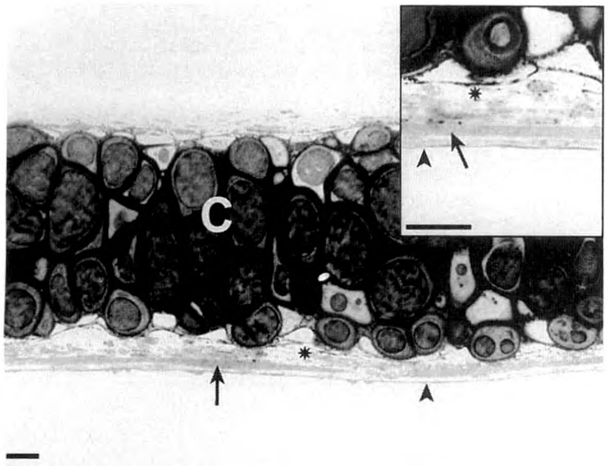

Fig 2. Light microscopy photograph of tympanic membrane after implantation of hydroxylapatite middle ear prosthesis with cartilage interposition (C). There are no signs of protrusion or extrusion of prosthesis. No sign of atrophy of middle, fibrous layer (arrow) is observed. Neither outer epidermal layer (arrowhead) nor inner, mucosal layer (asterisk) is affected. Bar — 20 μm; inset bar — 20 μm.

some signs of degradation, but all had an intact extracellular matrix.

The difference in the rates of protrusion or extrusion in group A (no cartilage) and group B (cartilage interposition) is statistically significant (χ^2; $p < .001$).

TRANSMISSION ELECTRON MICROSCOPY

Control Group. The tympanic membranes of the 3 left control ears showed an outer, epidermal layer, an inner, mucosal layer, and a middle, fibrous layer on TEM (Fig 3). The fibrous layer consisted of 2 types of fibers: the outer, radial fibers and the inner, circular fibers. The outer, radial layer, which was by far the largest, was arranged in radially oriented fibers and was about 2 to 3 μm thick. The inner, circular layer was much thinner. The fibers of both layers consisted of very thin (approximately 20 nm thick) fibrils, which did not seem to display the typical collagenous crossbanding patterns; this finding is in accordance with data in the literature.[19,20] Few interruptions of the (radial) fibers were observed. Some cells were present in the middle layer between the fibers. In 1 specimen, which was close to the malleus, the lamina propria of the epidermal layer showed cell-to-cell contacts, and many small vesicles were present underneath. The radial fibers at that location were loose, and many cells were observed between them.

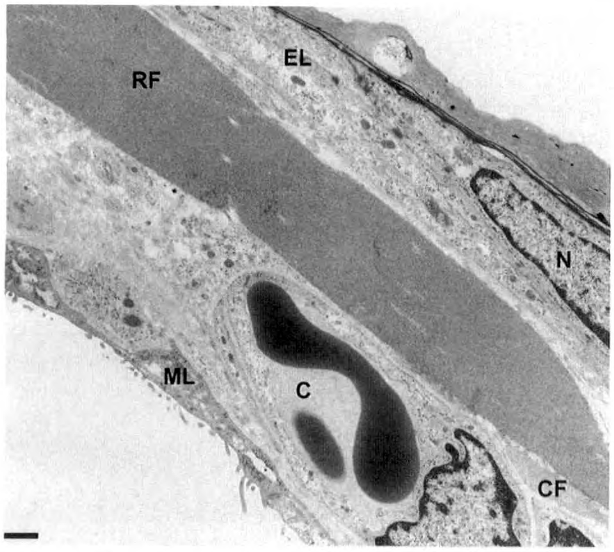

Fig 3. Transmission electron microscopy photograph of tympanic membrane of control ear without intervention. Tympanic membrane is built up of outer, epidermal layer (EL); middle, fibrous layer consisting of radial fibers (RF) and smaller, circular fibers (CF); and inner, mucosal layer (ML). N — nucleus; C — capillary; bar — 1 μm.

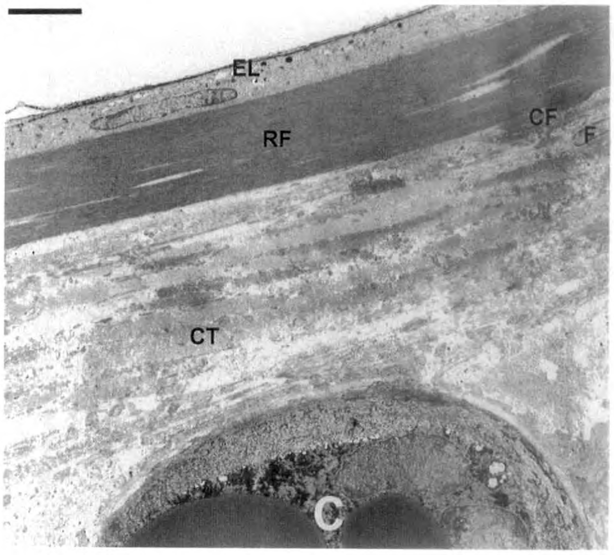

Fig 4. Transmission electron microscopy photograph of tympanic membrane after implantation of hydroxylapatite middle ear prosthesis with cartilage interposition (C). Aspect of outer, epidermal layer (EL) is normal. Middle, fibrous layer has normal appearance, with radial fibers (RF) and smaller, circular fibers (CF) still intact. Inner mucosal layer is replaced by connective tissue (CT) containing fibrocytes (F — fibrocyte nucleus) surrounded by collagen. Bar — 5 μm.

Research Group. The group investigated consisted of 5 cases with cartilage interposition and 1 case without cartilage interposition. In all cases, LM examination revealed an apparently normal histologic aspect of the tympanic membrane, including the 2 cases with a questionably atrophic fibrous layer. All tympanic membranes showed a normal appearance of the epidermal layer on TEM (Fig 4). The mucosal layer was absent in all cases and was replaced by a connective tissue layer that connected the tympanic membrane with the underlying cartilage or prosthesis. The connective tissue layer contained fibrocytes and contained collagen fibers with the characteristic crossbanding pattern. The fibrocytes were the end result of an active process of remodeling of the tympanic membrane (fibroblasts) that had stabilized by this point. The basal lamina on the mucosal side was no longer recognizable in half of the cases; in the other half, it was partly intact. The middle, fibrous layer was present in all cases (Table 2). In 3 of the cases, the fibers were continuous, including the 2 cases that showed questionable atrophy of the fibrous layer on LM. The other 3 cases showed some intermissions. Apart from this finding, the histologic appearance of this layer was normal.

DISCUSSION

In this experimental study, a cartilage disk was interposed between a synthetic middle ear prosthesis and the tympanic membrane in guinea pigs to investigate its effect on the extrusion process of the implant. Two groups of guinea pigs were studied: a group with cartilage interposition between the implant and the tympanic membrane and a group without cartilage interposition. A control group was also included.

A synthetic middle ear implant that is shielded by cartilage can easily be extruded or become liable to be extruded when the cartilage is displaced or resorbed. Clinical and experimental evidence suggests that cartilage alone softens and is either absorbed or replaced by fibrous tissue.[6,14] Preservation with perichondrium on one side improves the viability of the cartilage graft.[6,14,22,23] All of our cartilage disks were covered with perichondrium on the side opposed to the tympanic membrane. Histologic examination of the interposed cartilage disks with a perichondrium covering showed no evidence of resorption. All of the interposed cartilage disks had an intact extracellular matrix. It is clear that neither extrusion of a ceramic implant nor liability to extrusion can be prevented with certainty by interposition of a cartilage disk. The role of the interface is particularly important in cases of relative eustachian tube insufficiency, because the negative pressure inside the middle ear can cause the head of the prosthesis to exert increased pressure against the tympanic membrane.[7-9] This pressure may interfere with the nutrition of the overlying tympanic membrane.

The mechanism of extrusion can be infection or inflammation, a foreign body reaction to the prosthesis material, or pressure necrosis of the tympanic membrane.[6-8] Of all our cases, we reported 1 case of extrusion as a result of inflammation. The other protrusions or extrusions were not related to an inflammatory process. No lymphocytic infiltration or foreign body giant cells were observed in any cases — a finding suggesting that no foreign body reaction to the hydroxylapatite material was involved. However, the histopathologic observations strongly indicated a process of pressure necrosis of the tympanic membrane. The most prominent changes indicating pressure necrosis of the tympanic membrane consisted of atrophy of the middle, fibrous layer or total absence of this layer. Abnormal pressure of the prosthesis on the tympanic membrane may have interfered with the nutrition of the overlying tympanic membrane. Cartilage interposition may have caused less pressure necrosis of the tympanic membrane than a synthetic prosthesis would have caused when in direct contact with the tympanic membrane.

CONCLUSION

In this experimental model, protrusion and extrusion of a hydroxylapatite middle ear prosthesis was largely prevented by interposition of a cartilage disk. Higher extrusion and protrusion rates and severe histopathologic changes of the middle, fibrous layer of the tympanic membrane were observed in the group without cartilage interposition than in the group with cartilage inserted. This finding means that autologous cartilage indeed provides a good interface material between two systems with different elastic modules. On the basis of our observations, we strongly recommend the interposition of an autologous cartilage disk between a hydroxylapatite middle ear prosthesis and the eardrum. Further clinical evaluation of these experimental results is needed, initially by means of an animal experimental study in which the eustachian tube is obstructed. Additional evaluation is needed in the human middle ear, including a study of the sound conduction mechanism after tympanoplasty with cartilage interposition between the prosthesis and the tympanic membrane.

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AVERAGED ELECTRODE VOLTAGES IN USERS OF THE CLARION COCHLEAR IMPLANT DEVICE

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Averaged electrode voltages (AEVs) are of secondary importance for integrity testing of cochlear implant devices featuring back-telemetry. However, AEVs are device-independent and may show intermittent failures and deviant stimulation patterns unnoticed by telemetry. We collected AEVs from 18 users of the Clarion 1.2 system and 6 users of the HiFocus system in order to establish norms for evaluating AEVs in difficult cases. The stimuli were presented with the standard clinical software. Monopolar stimulation at about 16 μA showed large AEVs (mean, 173 μV) suitable for integrity testing. No electrode failures were found. The AEV amplitudes from neighboring electrodes differed by less than 30% (2 SD). The AEVs from subjects with the Clarion HiFocus electrode and/or the Clarion electrode positioner were within the normal range. The AEV amplitudes from bipolar stimulation were much more variable. Inversion of phases between electrodes was found in patients with an altered state of the cochlea (otosclerosis and osteogenesis imperfecta) and in a patient with a curled electrode tip. There was no correlation across subjects between AEVs and electrode impedances. Therefore, impedances are dominated by the electrode-tissue interface, in contrast to AEVs, which are determined by the volume conduction in the body.

KEY WORDS — averaged electrode voltages, cochlear implant, device failure, integrity testing.

INTRODUCTION

Back-telemetry circuits available in currently produced cochlear implant systems enable a simple means to test the integrity of the receiver electronics, as well as make an estimation of electrode impedances. In older systems, surface potentials of the electrical stimulus artifact have to be recorded. When averaged, these surface potentials are called averaged electrode voltages (AEVs), and the relationship between AEVs and electrode failures has been well documented for the Cochlear Nucleus Mini System 22.[1-9] In general, failures that were unequivocally established from behavioral threshold levels and comfort levels also were visible as deviant AEV patterns.[2] In children, however, a less than perfect match was found.[4] The AEV amplitudes vary widely among subjects, partly because of insertion depth,[2] but are a stable "fingerprint" of the individual current spread within and outside the cochlea if stimulus and recording parameters are optimized. In the absence of an external reference electrode, the largest AEVs for medial and apical electrodes are found in the Mini System 22 if the reference for stimulation is fixed to a basal electrode (known as pseudomonopolar coupling), and this has been proposed as the optimal stimulation mode.[2] Small and distorted AEVs are commonly found for apical electrodes with closely spaced bipolar stimulation and for medial electrodes in common ground electrode coupling. In pseudomonopolar mode, large amplitudes well above the noise level with minimal averaging can be picked up with recording electrodes at the ipsilateral and contralateral mastoid bones, although slightly higher amplitudes may be found with electrodes at the vertex and the ipsilateral mastoid bone.[9]

Typically, AEV amplitudes in pseudomonopolar coupling increase monotonically with the distance between the active and the reference electrodes. However, in cochleas deafened by otosclerosis, the phase of the artifact follows the orientation of the dipole, resulting in phase inversions across electrodes.[3] The deviant pattern in otosclerosis can be explained by means of a simple volume conduction model by varying the resistivity of the cochlear capsule (and skull) relative to that of the weak parts of the head.[10]

Although AEVs are sometimes considered obsolete, there are still reasons to record them in individual cases. First, they provide a device-independent means to test the integrity and stability of the implanted electronics and the electrode contacts even if the telemetry circuits are malfunctioning. Furthermore, inspection of the ongoing artifact signal shows intermittent failures — for instance, as a function of subcutaneous movement of the receiver — that may go unnoticed by the telemetry routine. Third, AEVs are potentially useful in identifying electrodes that disrupt normal tonotopy or cause side effects, especially in cases in which electrode insertion was difficult, in malformed cochleas, and in highly permeable cochleas (eg, cases of otosclerosis).

In this study, normative data were collected to as-

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PATIENT DATA OF 18 USERS OF CLARION ENHANCED BIPOLAR DEVICE AND 6 USERS OF HIFOCUS DEVICE

Patient No.	*Cause*	*Time From Onset of Deafness to First Fitting*	*Device*	*Clarion Electrode Positioning System*	*Preferred Strategy*	*Facial Nerve Stimulation in Bipolar Mode*	*Tonotopic Order in Monopolar Mode*
N100	Unknown	10 y	EB	No	CIS	DNT	Normal
N102	Unknown	12 y 1 mo	EB[a]	No	CIS	–	Electrodes 1-3 too high
N104	Trauma	2 y 3 mo	EB	No	CIS	–	Normal
N105	Enlarged aqueduct	8 mo	EB	No	SAS	–	Not reliable
N106	Progressive	2 y 3 mo	EB	No	CIS	DNT	Electrodes 7 and 8 too low
N108	Unknown	32 y 4 mo	EB	No	CIS	–	Normal
N111	Trauma	3 y 4 mo	EB	No	CIS	DNT	Normal
N115	Cogan's syndrome	1 y 1 mo	EB	No	CIS	–	Normal
N122	Progressive	16 y 1 mo	EB	No	CIS	Electrode 6[b]	Normal
N123	Viral infection	5 y 4 mo	EB	No	CIS	–	Normal
N124	Progressive	16 y 1 mo	EB	No	CIS	–	Normal
N143	Meniere's disease; otosclerosis?	7 y 3 mo	EB	No	CIS	Electrodes 2 and 3	Flat
N152	Unknown	28 y 7 mo	EB	No	CIS	–	Normal
N153	Unknown	5 y 8 mo	EB	No	SAS	–	Normal
N161	Unknown	5 y 3 mo	EB	Yes	SAS	–	Unreliable
N167	Otosclerosis	9 y 4 mo	EB	Yes	SAS	All at ULL	Flat to normal
N182	Viral infection	3 y 7 mo	HiFocus	Yes	SAS	–	Normal
N188	Unknown	15 y 1 mo	HiFocus	Yes	PPS	–	Normal
N192	Otosclerosis	6 y 1 mo	HiFocus	Yes	SAS	–	Normal
N197	Progressive	2 y 2 mo	HiFocus	Yes	SAS	–	Normal
N203	Progressive	6 mo	HiFocus	Yes	SAS	–	Electrode 1 too high
N206	Unknown	29 y 5 mo	HiFocus	Yes	PPS	–	Normal
N129	*Meningitis*	*18 y 8 mo*	*EB*[c]	*No*	*CIS*	–	*Not reliable*
N133	*Osteogenesis imperfecta*	*26 y 5 mo*	*EB*[d]	*No*	–	*Electrodes 1-7*	–[d]

Data from patients N129 and N133 were excluded from normative data, because of incomplete insertion and abnormal electrical conductivity of cochlea due to cause of deafness.

EB — Enhanced Bipolar 1.2 devices with medial contacts more apical than lateral contacts in all cases, CIS — continuous interleaved sampling, DNT — did not test, SAS — simultaneous analog stimulation, ULL — uncomfortable loudness level, PPS — paired pulsatile sampler.

[a] Postoperative radiographs confirmed tip to be curled.

[b] Pain behind ear.

[c] Partial insertion of electrodes 1 through 7, of which electrodes 6 and 7 are in drill-out.

[d] Radiographs showed array to be coiled back into internal auditory meatus; no profitable hearing sensations were elicited.

sist in the interpretation of individual AEV recordings from users of the Clarion device. Fifteen subjects used the Clarion Enhanced Bipolar 1.2 (EB 1.2, or precurved) device, and 6 used the Clarion HiFocus device. All HiFocus devices except 1, and 2 of the EB 1.2 devices, were inserted in combination with the "electrode positioning system," intended to bring the array in close contact with the medial wall of the scala tympani. At the outset, none of the subjects were identified as having an electrode failure. The AEVs were recorded from monopolar (with an extracochlear reference electrode) and bipolar stimulation on neighboring electrodes. These are the two coupling modes available in the clinical programming software.

In addition, the AEVs were compared with the electrode impedances. Impedances are affected by properties of the electrode-tissue interface and the volume conduction between the stimulating electrodes; in the case of perfect current sources, AEVs are independent of the voltage drop across the electrode-tissue interface. If impedances and AEVs are uncorrelated, the implication would be that impedances are dominated by electrode surface properties and that AEVs provide additional information not already contained in impedances.

METHOD

The AEVs were collected from 21 adult users of the Clarion system (see Table). Data collection was

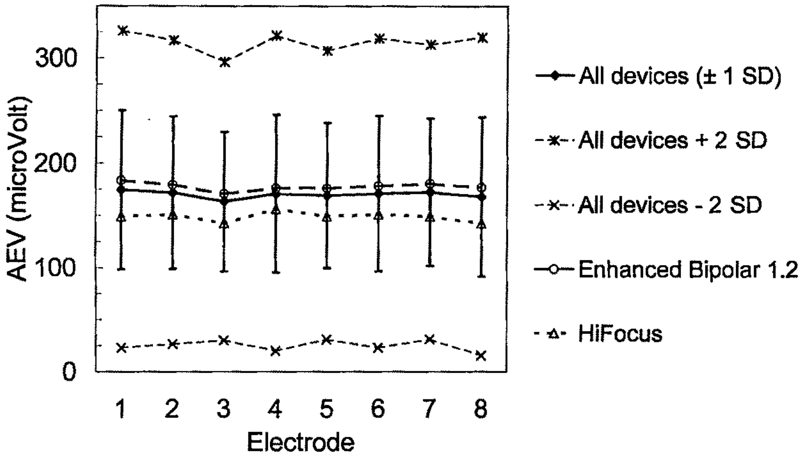

Fig 1. Averaged electrode voltages (AEVs) from monopolar medial coupling in 22 users of Clarion cochlear implant. Separate lines indicate mean AEVs for patients with Enhanced Bipolar 1.2 electrode array (n = 16) and HiFocus array (n = 6). Stimulation was at about 16 μA (16 current units). Electrode 1 is most apical one.

performed at least 3 months after surgery. All subjects but N133 used their devices consistently. Insertion of the electrode array was uneventful in all patients, with the exception of N129, in whom the basal turn had to be drilled out. The round window was filled with fascia and fibrin glue in all patients. Data from patients N129 and N133 were not included in the normative analysis of AEVs from monopolar stimulation (Figs 1 and 2).

The standard fitting software (SCLIN98) was used in electrically evoked auditory brain stem response mode to produce a biphasic pulse (300 μs per phase) at a rate of 203 pulses per second. For monopolar stimulation, "medial" contacts were chosen, which are the even-numbered contacts in the HiFocus electrode array. In monopolar mode, a stimulation level of 16 clinical units was chosen (approximately 16 μA), and 304 clinical units was used in bipolar mode. If the patient did not tolerate this level, stimulation was at the most comfortable loudness level, and AEVs were multiplied by 304 divided by the current level used. Silver–silver chloride recording electrodes with a contact area of about 1 cm^2 were applied to the patient's skin after rubbing with an abrasive gel. Recordings were made between an electrode on the mastoid bone on the side of the implant (positive) and an electrode on the contralateral mastoid bone (negative). An indifferent electrode was placed on the patient's arm. The recording was triggered from the trigger unit produced by Advanced Bionics. The number of averages varied between 125 for monopolar stimulation and 1,024 for bipolar stimulation. The amplifier gain was typically set to 50 μV for monopolar stimulation and 20 μV for bipolar stimulation. The amplifier had a passband of 100 to 6,000 Hz and an input impedance of 100 MΩ per 15 pF. The 100-Hz high-pass frequency was chosen to minimize biological noise and line noise in the signal. In tank measurements with the Clarion electrode in saline solution, it was established that a cutoff frequency of 100 Hz, as opposed to 30 Hz, led to a difference in peak-to-peak amplitude of less than 10%, with a similar small effect on the amplitude of the first phase compared to the second phase. A passive low-pass resistor-capacitor network (cutoff frequency at 350 kHz) was applied to the measuring leads to prevent saturation of the amplifier by distortion products of the transmitter radiofrequency signal. The signal was sampled for 10 ms with a 3–dB per octave high-frequency cutoff at 6 kHz. The reported amplitude is the peak-to-peak amplitude or the baseline-to-peak amplitude, whichever was larger. Positive values are assigned to positive-leading AEVs from monopolar stimulation. The relative orientation of the two contacts in a bipolar pair is opposite for the EB 1.2 device as compared to the HiFocus device. Positive values were assigned to negative-leading bipolar AEVs in the case of an EB 1.2 device, and negative values were assigned in the case of a HiFocus device.

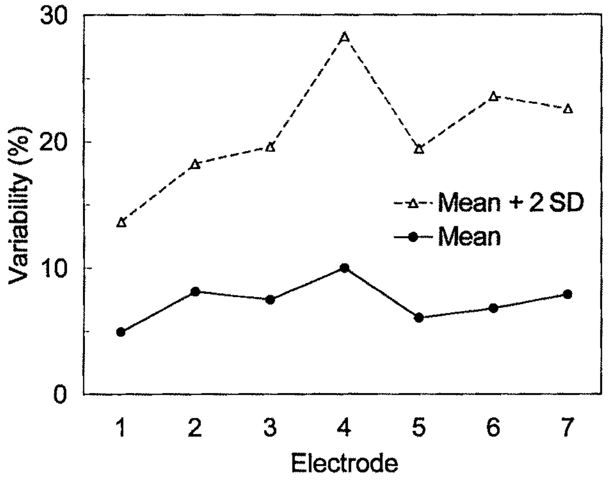

Fig 2. Variability of AEVs across electrode array when used to assess suspect electrodes in individual patients. For each subject, 7 differences of AEVs from neighboring electrodes were calculated. Lines are differences averaged across subjects. Monopolar coupling (n = 22 users; see legend for Fig 1).

Electrode impedances were measured with the

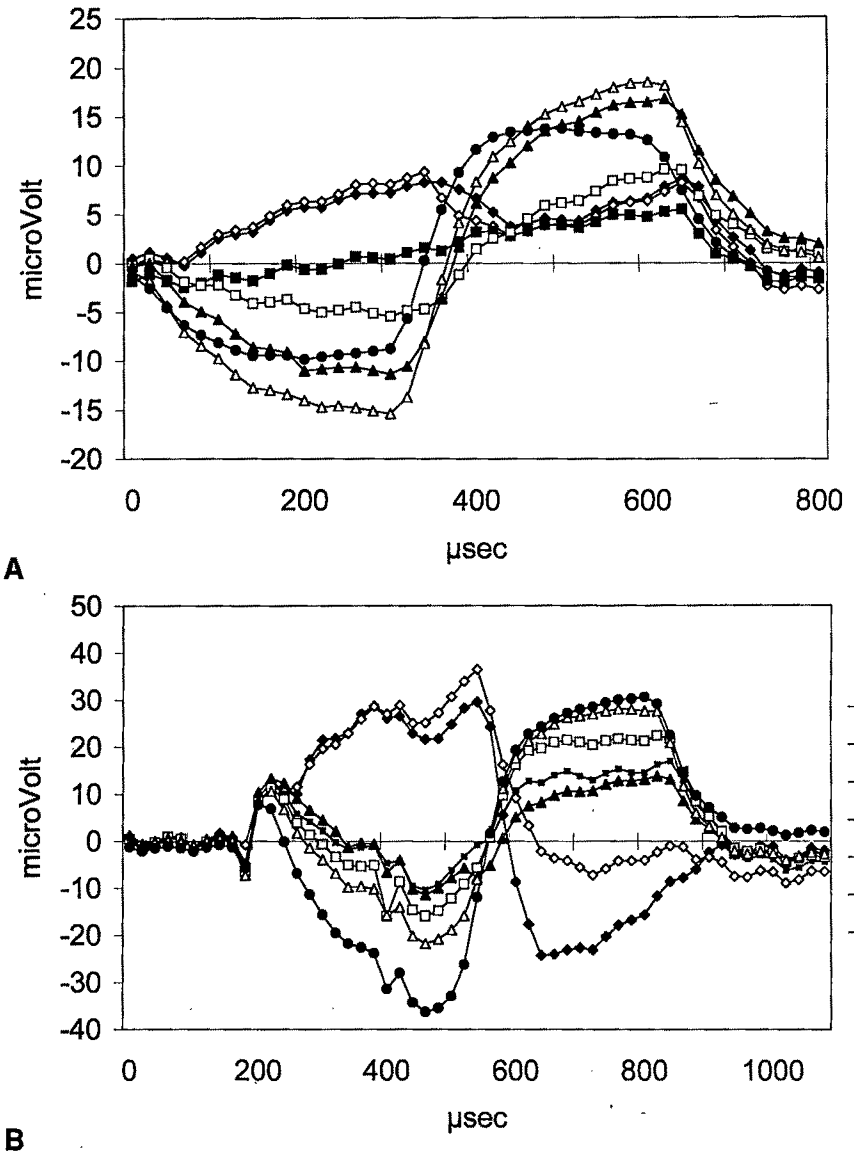

Fig 3. A) Typical (nondeviant) waveforms from bipolar stimulation classified as showing nonalternating polarities, even from apical electrodes. B) AEVs classified as deviant, with inverted polarities for apical compared to basal electrodes, in case of otosclerosis.

standard clinical software, which uses a 1-kHz sinusoidal stimulus at less than 10 µA. Monopolar medial coupling was used, in which the medial ball electrode was coupled to the receiver case.

RESULTS

The AEVs from monopolar stimulation showed a biphasic waveform with identical polarity in all patients and all electrodes. The mean peak-to-peak amplitude at a current level of about 16 µA was 173 µV. An analysis of variance with the electrode as the repeated variable showed no difference in AEVs between the EB 1.2 device and the HiFocus device (F = 0.81, p = .38). In Fig 1, the means and SD per electrode are shown, as well as the means per device. The AEVs did not differ between electrodes (F = 0.04, p = .96). The recordings of some individual patients, however, do show a gradual increase or decrease in amplitude across the array. Therefore, as a measure of the variability of AEVs across individual electrode arrays, the AEV from each electrode was subtracted from that of its apical neighbor. The absolute differences averaged across subjects ranged from 4.9% to 10.5% (Fig 2). Differences between AEVs and neighboring electrodes of greater than 30% exceed the largest value of the mean plus 2 SD and therefore indicate a possible failure.

The bipolar recordings showed great variability across electrodes in terms of waveform morphology. Individual waveforms were assigned a positive amplitude if the second phase was more positive than the first. This rule was introduced because of the many nonreversing (bifid) waveforms that were encountered. Figure 3A shows a typical waveform series from an EB 1.2 device. Positive values were assigned to all amplitudes in this series. Strictly speaking, electrode 2 produced a slightly higher amplitude for the first phase than for the second phase, but

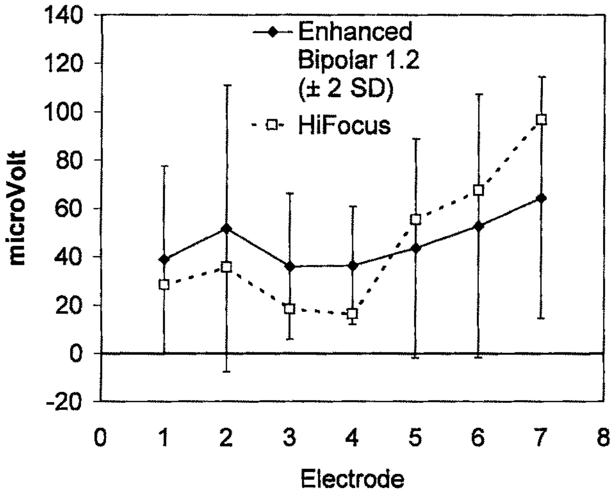

Fig 4. Mean AEVs from bipolar stimulation for all cases without inverted polarities on any electrodes. For Enhanced Bipolar implants, mean ± 2 SD is shown.

the amplitude was still considered positive in light of the very similar pattern on electrode 1. Positive amplitudes for all electrodes (no inversions across electrodes) were found in 14 subjects. The mean amplitudes found for these subjects are presented in Fig 4. The amplitudes were smallest for electrode 4. In 5 subjects, the phases were clearly inverted for the apical electrodes (for example, see Fig 3B), and those amplitudes were entered as negative; all cases are presented in Fig 5. Patient N102 showed inverted polarities on electrodes 1 and 2. A postoperative radiograph showed the tip of the electrode array to be curled, and the tonotopy was accordingly irregular for the apical electrodes. Both patients N105 and N133, who had known cochlear malformations, showed inverted polarities for the apical electrodes. (In patient N133, the array was involuntarily placed in the internal auditory meatus, as well.) Patient N129 (partial drill-out) showed very large amplitudes on the medial electrodes and inverted polarity on the most basal electrode. Patient N167 showed inverted polarities on medial electrodes 2 to 5, in accordance with earlier findings in patients deafened by otosclerosis.[3] Patient N143, although also deafened by otosclerosis, showed inverted polarities only on electrode 3, which produced facial nerve stimulation in bipolar mode together with electrode 2.

The mean AEV from monopolar stimulation did not show a relationship with mean electrode impedance (Fig 4, EB 1.2 devices without positioner only). In addition, a correlation was calculated between AEV amplitude and impedance for each user of the EB 1.2 device. In none of the subjects was the correlation significant at the 1% level.

Similarly, the mean AEV per subject did not correlate significantly with the mean subjective threshold in monopolar mode ($R^2 = .001$, the data of outlier patient N106 excluded). In none of the subjects was the correlation significant at the 1% level. Similarly, the mean AEVs and thresholds across subjects did not correlate significantly ($R^2 = .0005$).

DISCUSSION

Monopolar stimulation at a very low current level showed large AEVs suitable for the analysis of device integrity. At 16 clinical units, the mean amplitude was 173 μV (SD, 75 μV), which is large enough to be evaluated in the running signal in quiet subjects. As such, open contacts, even intermittent, can easily be detected even if telemetry circuits are not functioning. In principle, short-circuited electrode leads from different current sources will only result in deviant waveforms if a connection to the ground electrode is made. This may not always be the case. The variability of the AEVs between neighboring electrodes (2 SD) was less than 30%, which can be used as a rule to identify outlying amplitudes. Another application of AEV recording is to differentiate physiological from technical instabilities in cases in which electrode impedances are relatively con-

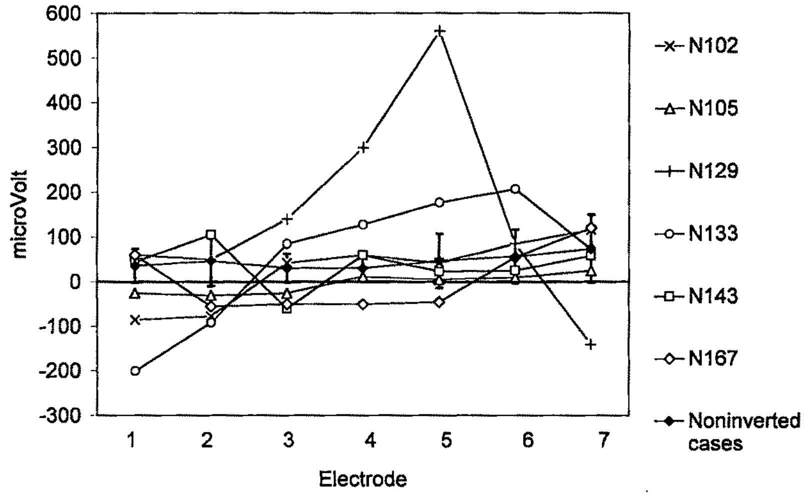

Fig 5. AEVs from bipolar stimulation in 6 cases that showed inverted polarities.

stant and AEVs might show an altered current distribution (eg, electrode slippage). However, such an analysis would require a baseline recording before any incidents. As such cases are rare, recording of AEVs in all patients does not seem to be a necessary part of postoperative management. In bipolar mode, higher stimulus levels were needed for an adequate signal-to-noise ratio. The AEVs from the apical channels were small and showed marked differences between patients that were unrelated to device integrity. Therefore, monopolar stimulation is the preferred mode for detecting electrode failures.

The AEVs from bipolar stimulation showed inverted polarities in 2 of 3 patients deafened by otosclerosis and in patient N133, deafened by osteogenesis imperfecta. Mens et al[3] also found deviant AEVs in similar cases with an abnormal structure of the cochlea by using wide bipolar and common ground stimulation modes. In addition, inverted polarities were found in a patient with a large vestibular aqueduct, indicating abnormal current pathways, as in otosclerosis. As in the earlier study,[3] no conclusive relationship between the occurrence of facial nerve stimulation and deviant AEVs was found. Bipolar stimulation did produce deviant AEVs (with inverted polarity) on electrodes that a radiograph had shown to be curled backward (patient N102).

No relationship was found between electrode impedance and AEV amplitude. Thus, AEVs provide independent information on the volume conduction in the head that is not contained in electrode impedances. To understand how surface potentials can be unrelated to the electrode impedances, it has to be noted that 1) current sources are used that attempt to stimulate at a constant current level irrespective of the impedance and 2) the voltage drop at the electrode-tissue boundary by itself does not affect the current pathway through the body. Apparently, the overall impedance is dominated by the impedance at the electrode-tissue boundary, instead of the impedance of the intervening tissues. Patients using the EB 1.2 device with the electrode positioner (2 patients) and patients using the HiFocus electrode array with a positioner (6 patients) showed AEVs within the range of the EB 1.2 electrode array without a positioner, again illustrating the dissociation between near-field factors (eg, electrode characteristics) and far-field effects.

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DOES ENDOGENOUS OR EXOGENOUS ADENOSINE FACILITATE THE FUNCTIONAL RECOVERY OF THE COCHLEA AFTER ISCHEMIA?

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The present study was undertaken to determine whether adenosine attenuates cochlear dysfunction induced by transient ischemia. Adenosine or erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an adenosine deaminase inhibitor, was administered by perilymphatic perfusion to albino guinea pigs that were subjected to cochlear ischemic episodes of 30-minute duration. The threshold shift of the compound action potential (CAP) from the preischemic value was significantly reduced in the animals perfused with EHNA 1 hour after the onset of reperfusion. However, perfusion of adenosine at concentrations of 100 μmol/L to 10 mmol/L did not reduce the postischemic CAP threshold shift by either 1 hour or 4 hours after the onset of reperfusion. These results suggest that the elevation of the adenosine concentration did not exert a protective effect on the cochlear ischemia-reperfusion injury, and that the protective action of EHNA is unrelated to elevating the adenosine concentration.

KEY WORDS — adenosine, adenosine deaminase, cochlea, cochlear blood flow, compound action potential, ischemia.

INTRODUCTION

The intracellular concentration of adenosine triphosphate (ATP) is regulated by a dynamic balance between ATP synthesis and utilization. Ischemia inhibits the synthesis of ATP and induces its rapid catabolism, with accumulation of adenosine diphosphate, adenosine monophosphate, and adenosine.[1] Adenosine is, in turn, degraded to inosine by adenosine deaminase (ADA), and inosine is further degraded to hypoxanthine.[1] It has been shown in many different tissues and cells that inhibition of ADA is a potent way to elevate the extracellular concentration of adenosine when ATP degradation is accelerated during ischemia.[2-5]

Adenosine is a potent vasodilative and cardiodepressive agent that is thought to act as a neuromodulator in the central nervous system. The protective properties of adenosine in myocardial and cerebral ischemia have been demonstrated in several studies.[6-8] Earlier experiments[9] reporting that selective activation of A1 adenosine receptors alleviated cochlear dysfunction induced by transient ischemia suggest that adenosine may ameliorate ischemia-induced cochlear dysfunction. The present study was designed to examine the effects of adenosine on the cochlear dysfunction induced by transient ischemia. Toward this end, exogenous adenosine at several concentration levels was infused into the cochlea after an ischemic episode to assess the protective effects of this compound. Additionally, to manipulate the levels of endogenous adenosine, several concentrations of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an ADA inhibitor, were infused in another set of animals. In this manner, the protective role of adenosine with respect to preserving cochlear function under postischemic conditions was systematically examined.

MATERIALS AND METHODS

Animal Preparation. Forty-six albino guinea pigs (300 to 400 g) with a normal Preyer's pinna reflex were anesthetized with intraperitoneal injections of pentobarbital sodium (28 mg/kg, Nembutal, Abbott). After a tracheostomy was performed, the animals were artificially ventilated with room air. Catheters were inserted into the external jugular vein to replace fluids, and into the right carotid artery to record blood pressure. An electrocardiogram and rectal temperature, which was maintained at 37°C ± 1°C, were monitored throughout the experiment. The care and use of the animals used in this study were approved by the Animal Experiment Committee at the University of Tsukuba.

Measurement of CAP and Induction of Transient Ischemia. The methods to measure the compound action potential (CAP) and to induce transient cochlear ischemia have already been described elsewhere.[10,11] In brief, the CAP was recorded with a silver–silver chloride electrode at the bony edge of the round window. The reference electrode was placed on the neck muscles. The responses were evoked by tone bursts

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from 2- to 16-kHz pure tones (rise-decay time, 1 ms; duration, 10 ms) delivered every 200 ms in an open-field system. Thirty-two, 64, or 128 consecutive potentials were amplified, summed, and averaged by a signal processor (NEC Medical Systems, Synax 2100). The sound pressure level (SPL) required to evoke a 10-µV CAP was defined as the threshold. When sounds of 110 dB SPL failed to evoke a 10-µV CAP, the threshold was defined as 115 dB SPL to simplify calculations.

The anterior inferior cerebellar artery and the labyrinthine artery were exposed via the skull base. The cochlear ischemia-reperfusion condition was obtained by pressing and releasing the labyrinthine artery at the porus acusticus internus with a metal rod.[10] A laser-Doppler flowmeter (BRL-100, Bio Research Center) was used in order to monitor cochlear blood flow (CBF). The CBF was plotted at 10-minute intervals as a percentage relative to the value just prior to the onset of perilymphatic perfusion, and the postmortem value was set at 0%.

Perilymphatic Perfusion. The cochlea was prepared for perilymphatic perfusion by making 2 holes. The inlet hole was made in the scala tympani of the basal turn, and the outlet hole in the apex. Artificial perilymph,[12] consisting of 137 mmol/L sodium chloride, 5 mmol/L potassium chloride, 2 mmol/L calcium chloride, 1 mmol/L magnesium chloride, 1 mmol/L sodium bicarbonate, and 11 mmol/L glucose, at a pH of 7.4, was perfused at a rate of 2.5 µL/min. Adenosine or EHNA was dissolved in the artificial perilymph solution, and the pH was adjusted to 7.4 as required.

Effects of Perilymphatic Perfusion of Adenosine or EHNA on CAP Threshold. Before ischemia studies were conducted, the effects of adenosine or EHNA on the CAP threshold were examined on 6 untreated animals. Artificial perilymphs containing 10 mmol/L adenosine (n = 3) and 100 µmol/L EHNA (n = 3) were perfused for 4 hours and 1 hour, respectively. The CAP thresholds were measured before and after the perfusion, and the values obtained after the perfusion were compared with the preperfusion values.

Effects of Adenosine and EHNA on CAP Threshold Shifts After Transient Ischemia. Forty guinea pigs subjected to a 30-minute ischemic episode were randomly assigned to 1 of the following 3 groups.

1. Artificial perilymph–perfused group (n = 5). Artificial perilymph was perfused from 10 minutes before the onset of ischemia to 4 hours after the termination of ischemia. The CAP thresholds were measured before starting the perilymphatic perfusion and at 1 hour and 4 hours after the termination of the ischemic episode.

2. Adenosine-perfused group (n = 15). Artificial perilymph containing 100 µmol/L, 1 mmol/L, or 10 mmol/L adenosine was perfused from 10 minutes before the onset of ischemia to 4 hours after the termination of ischemia (n = 5 in each subgroup). The CAP thresholds were measured before starting the perilymphatic perfusion and at 1 hour and 4 hours after the termination of the ischemic episode.

3. EHNA-perfused group (n = 20). Artificial perilymph containing 1, 10, 50, or 100 µmol/L EHNA was perfused from 10 minutes before the onset of ischemia to 1 hour after the termination of ischemia (n = 5 in each subgroup). The CAP thresholds were measured before starting the perilymphatic perfusion and at 1 hour after the termination of the ischemic episode.

The postischemic CAP threshold shifts were compared between the adenosine- and EHNA-perfused groups and the artificial perilymph–perfused group at 1 hour and/or 4 hours after the termination of ischemia.

Statistical Analysis. All data are expressed as mean ± SD. Comparison of CBFs and mean arterial blood pressures was made by repeated-measures analysis of variance (ANOVA). Comparison of the CAP thresholds was made by a 2-way ANOVA followed by a Scheffé test as required. A p value of less than .05 was considered statistically significant.

RESULTS

Mean Arterial Blood Pressure and Cochlear Blood Flow. The mean arterial blood pressure remained stable throughout the experiments in all experimental groups, and there was no change in the arterial blood pressure with perfusion of adenosine or EHNA (data not shown).

Figure 1 shows the response of CBF to the application of adenosine or EHNA. In all experimental groups, the CBF decreased rapidly when the labyrinthine artery was pressed and remained at an almost constant level during ischemia. When the artery was unclamped, the CBF promptly increased with a transient overshoot and then gradually regained a stable level. As shown in Fig 1A, perilymphatic perfusion of adenosine at a concentration of either 100 µmol/L (open circles) or 1 mmol/L (open triangles) did not affect the CBF. Although 10 mmol/L adenosine (open squares) increased the CBF during the preischemic and postischemic periods, this effect was not statistically significant.

The CBF was not significantly affected by perfusion of EHNA, as shown in Fig 1B, regardless of concentration.

Effect of Adenosine or EHNA on CAP Threshold

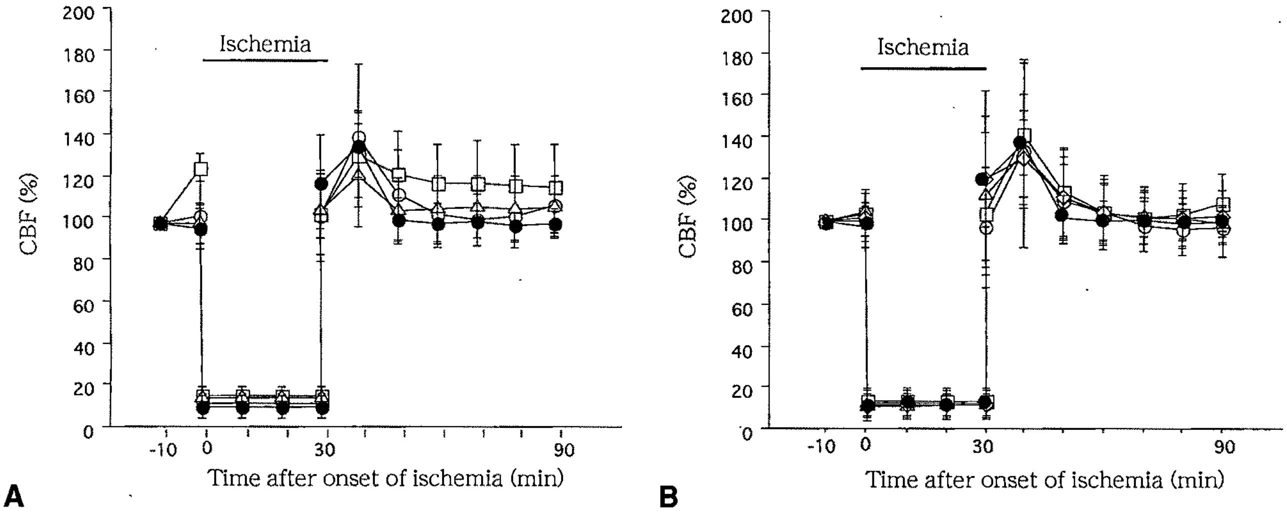

Fig 1. Cochlear blood flow (CBF; percent) before, during, and after onset of ischemia. **A)** Adenosine-perfused group versus control group. ● — control group; ○ — 100 µmol/L subgroup; Δ — 1 mmol/L subgroup; □ — 10 mmol/L subgroup. **B)** Group perfused with erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) versus control group. ● — control group; Δ — 1 µmol/L subgroup; □ — 10 µmol/L subgroup; ◊ — 50 µmol/L subgroup; ○ — 100 µmol/L subgroup.

Shifts. As shown in Fig 2, perilymphatic perfusion of 10 mmol/L adenosine for 4 hours (Fig 2A), or 100 µmol/L EHNA for 1 hour (Fig 2B), did not significantly affect CAP thresholds.

As shown in Fig 3, adenosine did not alleviate the postischemic CAP threshold shifts at either 1 hour or 4 hours after the onset of reperfusion. In contrast, as shown in Fig 4, 50 (open inverted triangles) or 100 (open squares) µmol/L EHNA significantly decreased ($F = 11.4$, $p < .01$) the postischemic CAP threshold shifts as compared with the artificial perilymph–perfused control group (closed circles) at 1 hour after the onset of reperfusion, although 1 (open circles) or 10 (open triangles) µmol/L EHNA failed to affect the CAPs.

DISCUSSION

In the present study, a laser-Doppler flowmeter was used to monitor CBF. The CBF decreased rapidly when the labyrinthine artery was pressed, remained at an almost constant level during ischemia, and promptly increased with a transient overshoot upon unclamping of the vessel. These findings are in close agreement with those of previous ischemia studies

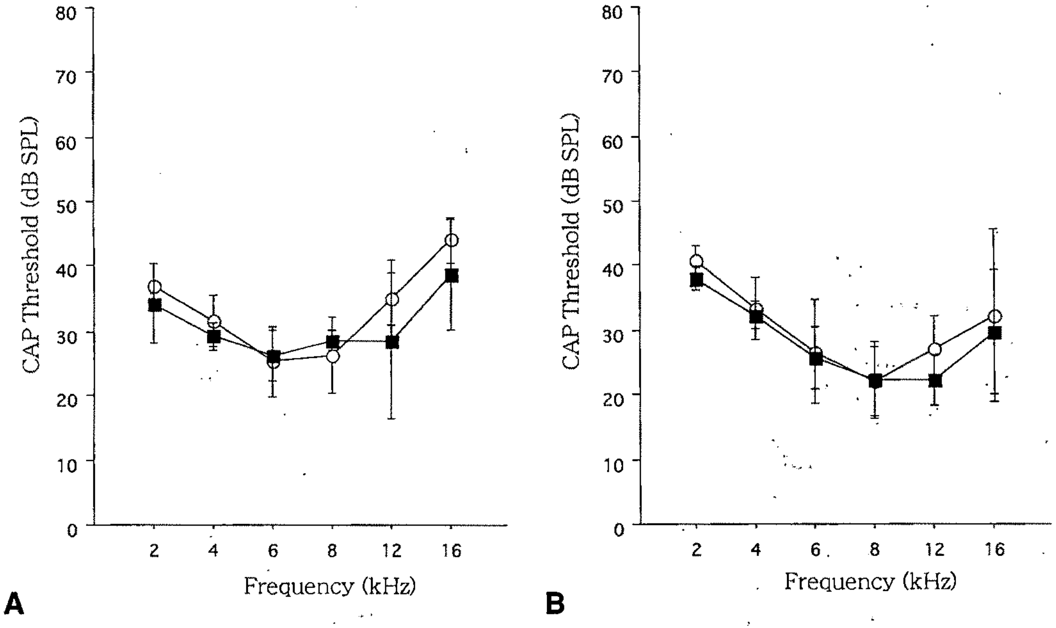

Fig 2. Compound action potential (CAP) thresholds before (filled squares) and after (open circles) perfusion of drugs. **A)** Effect of 4-hour perfusion of 10 mmol/L adenosine. **B)** Effect of 1-hour perfusion of 100 µmol/L EHNA.

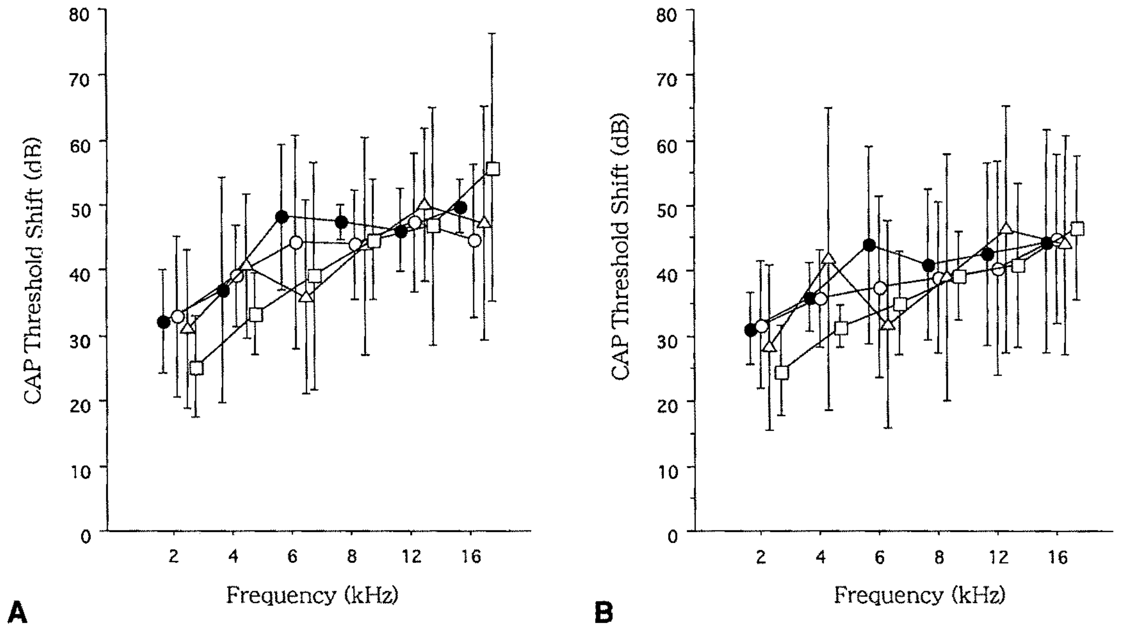

Fig 3. Effect of adenosine on postischemic CAP threshold shifts **A)** 1 hour and **B)** 4 hours after restoration of cochlear blood circulation. ● — control group; ○ — 100 µmol/L subgroup; Δ — 1 mmol/L subgroup; □ — 10 mmol/L subgroup.

of gerbils[13,14] and guinea pigs.[15]

Adenosine is normally present in the extracellular fluid, and its concentration dramatically increases during tissue ischemia.[4,5] Various studies have claimed that adenosine is protective against ischemia or hypoxia. Exogenous adenosine attenuated the hypoxic injury of cortical cell cultures and hippocampal slices.[6,8] In addition to the protective effects against cerebral ischemia in vitro, adenosine significantly ameliorated ventricular arrhythmias after myocardial ischemia.[7] These previously reported findings support the notion that adenosine is a possible candidate for ameliorating ischemia-induced cochlear dysfunction. However, in the present study, adenosine concentrations of 100 µmol/L to 10 mmol/L did not have any effect on the postischemic CAP threshold shifts.

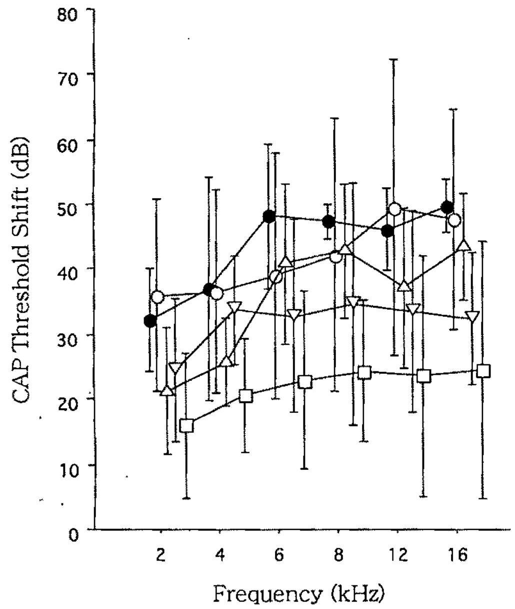

Fig 4. Effect of EHNA on postischemic CAP threshold shifts 1 hour after restoration of cochlear blood circulation. ● — control group; ○ — 1 µmol/L subgroup; Δ — 10 µmol/L subgroup; ∇ — 50 µmol/L subgroup; □ — 100 µmol/L subgroup.

The actions of adenosine are mediated by 4 different subtypes of adenosine receptors, namely, A1, A2a, A2b, and A3 receptors. The protective properties of A1 receptor agonists against ischemic injury have been demonstrated in several organs, including the cochlea.[9,16-18] However, activation of A2 or A3 receptors exhibited contradictory effects against ischemic damage, depending on the organ. For example, activation of A2 or A3 receptors was purported to exert protective effects in the cardiovascular system. The A2 receptor agonist CGS-21680 inhibited neutrophil activation[16] and reduced ischemia-reperfusion injury of the heart.[17,19] Furthermore, the A3 receptor agonists IB-MECA and CI-IB-MECA attenuated hypoxic or ischemic injury of the heart.[18,20] In contrast to the cardiovascular system, activation of A2 or A3 receptors is suggested to play a significant role in generation of ischemic damage of the central nervous system, although the precise mechanism has not been fully clarified.[21] Because exogenous adenosine did not ameliorate the postischemic CAP threshold shifts in the present study, there is a possibility that activation of cochlear A2 or A3 receptors has deleterious effects in cochlear ischemia-reperfusion injury, or nullifies the effects of activation of A1 receptors. Further studies are necessary to elucidate the precise pathological roles played by adenosine A2 and A3 receptors in cochlear ischemia.

In the present study, EHNA significantly ameliorated the postischemic cochlear dysfunction 1 hour after the onset of reperfusion. It has been shown in many different tissues and cells that inhibition of ADA is a potent way to elevate the adenosine concentration when ATP degradation is accelerated during ischemia.[2-5] However, it is highly likely that the protective mechanism of EHNA is not related to elevating the extracellular concentration of adenosine, at least in the cochlea, because exogenous adenosine did not exert protective effects at either 1 hour or 4 hours after the onset of reperfusion.

Some authors have claimed that EHNA protects organs against ischemia-reperfusion injury by inhibiting the formation of hypoxanthine, a substrate of xanthine oxidase, and thus by inhibiting the generation of free radicals.[22] Over the past decade, oxidative injury has been considered to be one of the central mechanisms of cochlear ischemia-reperfusion injury.[11,23] Xanthine oxidase has been proposed as one of the major sources of free radical generation induced by hypoxia or ischemia in the cochlea.[23,24] However, according to other findings, the protective effect of EHNA on cochlear dysfunction is not likely related to a decrease in hypoxanthine, because oxipurinol and allopurinol, potent xanthine oxidase inhibitors, failed to ameliorate cochlear ischemia-reperfusion injury at the concentration at which these inhibitors fully blocked xanthine oxidase activity.[25] The mechanism(s) of protection by EHNA must be investigated more thoroughly in future studies.

In conclusion, exogenous adenosine did not exert a protective effect on the cochlear ischemia-reperfusion injury induced in the present experiments. Although EHNA did exhibit a protective effect on the cochlea after transient ischemia, it is not likely that the mechanism of protection is related to an EHNA-induced elevation of the endogenous levels of adenosine.

ACKNOWLEDGMENT — We are grateful to Dr H. Takahashi for his advice in the statistical analysis.

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SOFTWARE REVIEW

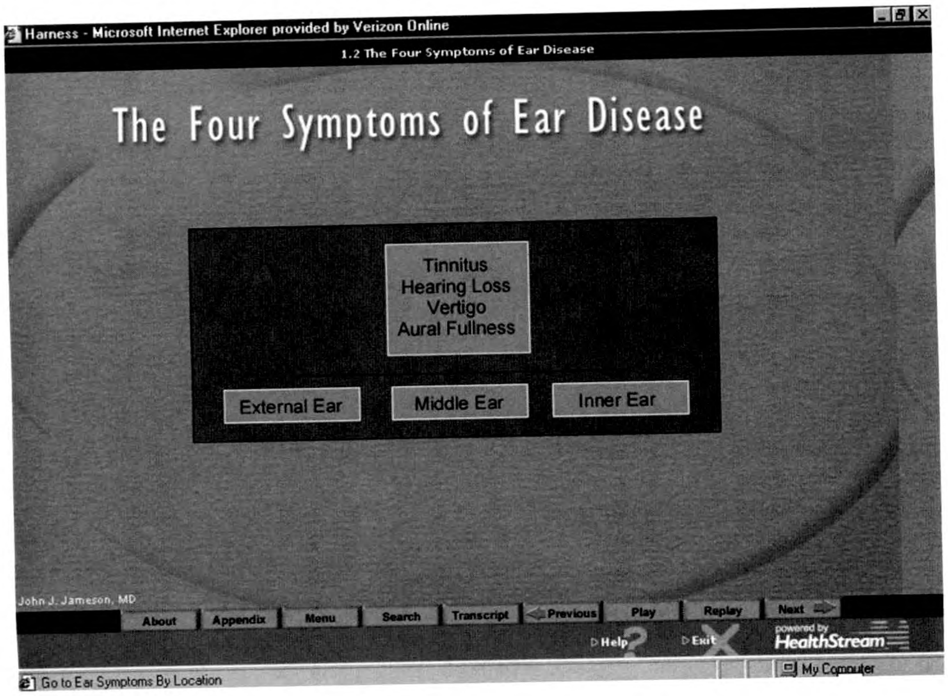
CORE CURRICULUM IN ADULT PRIMARY CARE 2001: OFFICE OTOLARYNGOLOGY AND OPHTHALMOLOGY

Required software: (Macintosh or PC computer) Windows 95, 98, NT, and 2000, Netscape 4.51, Adobe Acrobat Reader 4.0, Macromedia Shockwave 8, and Quicktime 4.1.2.
Course cost: The cost of the CME credits depends upon the quantity of courses purchased: 72 credits with a price of $649, 26 credits for $349, or a single CD-ROM for $99. The Core Curriculum Committee also has a web site with several on-line CME courses costing $25 per credit, and a few free courses funded by grants.

Core Curriculum in Adult Primary Care 2001 is a Continuing Medical Education (CME) program targeted to primary care. The CD-ROM that was reviewed covered subjects in office otolaryngology and ophthalmology; a total of 4 CME credits can be earned. There are a total of 10 lectures. The 5 lectures in otolaryngology were reviewed. Each lecture is composed of a number of slides ranging from 17 to 42 pages. Each page presents information and has the voice of a lecturer that is played while the physician views the slide (see Figure). Several pages are in Shockwave and have a degree of animation; however, most pages are text. There is also a written transcript of all lectures that can be viewed and printed.

The 5 primary care otolaryngology lectures are 1) tinnitus, 2) hearing loss, 3) benign positional vertigo, 4) allergic and nonallergic rhinitis, and 5) management of snoring.

All lectures present the educational material in a clear, precise, and interesting manner. I would highly recommend this lecture series. The only shortcomings I found were that there was no mention of referred symptoms of ear pain and pressure that are not of otologic origin, ie, temporomandibular joint syndrome; and the particle repositioning maneuver was taught with pictures rather than a video. I am also not sure about the statement that ear wax can cause vertiginous symptoms. There is only 1 video, which shows radiofrequency surgery for snoring. The depth of discussion on radiofrequency for the treatment of nasal disorders and snoring was aimed more at otolaryngologists than at primary care physicians,



Example of screen display.

with 18 slides and 1 video devoted to this subject. I feel this section would make an excellent short CME course for otolaryngologists.

The CD-ROM contains links to related web sites, but not to references, abstracts (posted on PubMed), or related on-line full-text articles. This would be an excellent addition.

A test that is graded by the computer is available on the CD. There are 24 multiple-choice and true-false questions for the otolaryngology lectures. After a question is answered, the physician is told whether he or she answered it correctly. The entire test (for both the ophthalmology and otolaryngology sections) must be taken; this will not be true for the on-line version. If a score of 70% or higher is achieved, a test form is printed and can be sent via US mail for accreditation. An improvement would be to allow the test to be sent via E-mail.

Many universities and professional organizations now offer on-line CME courses that are available to physicians. Costs vary, but some are free and funded by grants. Truly interactive courses with streaming video are rare, but as the speed of the Internet increases, these will become commonplace. So far, very few are designed for otolaryngologists.

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BOOK REVIEWS

Otitis Media in Infants and Children

Charles D. Bluestone and Jerome O. Klein. Third edition. Hard cover, illustrated, indexed, 404 pages, 2001. Saunders, Philadelphia, Pa, $85.

Purpose: "To provide a critical and contemporary review of the important and latest medical information that is of interest to all health care workers committed to the well being of infants and children." The material in this edition is based on investigation by the authors and a review of the literature as of January 1, 2000.

Contents: This book can be divided into 3 parts: 1) basics (definitions, anatomy, physiology); 2) causation (epidemiology, microbiology, immunology); and 3) diagnosis, treatment, and complications. The first edition was published in 1988, the second in 1995. Updating in this edition includes new classification and staging systems for otitis media and its complications. A classification includes eustachian tube dysfunction. There is an up-to-date portion on the method of clinical and microbiological diagnosis of otitis media. New information includes antibiotic resistance of otitic bacterial pathogens and the latest available information regarding viral and bacterial vaccines for prevention. Most important, it includes reprinting of some of the classic studies of the previous books.

Attributes: The following is quoted from Adam Politzer's last edition of the *Lehrbuch*, published in 1926: "Diseases of the middle ear originate in the mucus membrane lining; from here those inflammatory changes start which not only often disturb the hearing, but not infrequently extend to neighboring vital organs, and endanger the life of the patient. The knowledge of these changes is therefore of great importance as they form the basis for the diagnosis and treatment of middle ear infections." (Politzer A. Textbook of the diseases of the ear. 6th ed. Philadelphia, Pa: Lea and Febiger, 1926:270).

In the current volume, the authors have elaborated extensively on those objectives pointed out by Politzer approximately 75 or more years earlier. They have attempted to evaluate and discuss the various aspects of serous otitis media in a concise manner directly applicable to expeditious patient care.

At the time of this publication, Tauno Palva has published an essay whose thrust is to aerate or alleviate disease in the epitympanum in cases in which multiple tubes have been needed. Perhaps this essay of Palva should be reviewed by Bluestone and Klein to assess otitis media complications before a fourth edition.

Applicability: The book is of use to all physicians involved in the treatment of otitis media in infants and children, especially otolaryngologists, pediatricians, and family practitioners. It is another step toward better understanding and more accurate and effective treatment of the various aspects of otitis media.

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Decision Making in Ear, Nose, and Throat Disorders

Cuneyt M. Alper, Eugene N. Myers, and David E. Eibling. Hard cover, indexed, 385 pages, 2001. Saunders, Philadelphia, Pa, $95.

Purpose: To provide basic clinical information, in an algorithm format, that is relevant to otolaryngologists in making a diagnosis.

Contents: In addition to the anatomic sections, several other topics are covered that are important to the overall review of otolaryngology, including neck, plastics, and general subjects (bleeding disorders, anesthesia, and, to be exact, 11 more such general subjects).

Attributes and Limitations: Each lesson (topic such as dizziness) is structured so that it provides an explanation of a portion of the algorithm (designated by alphabetical letters that match in the algorithm and the page of explanation). Typically, the explanation part consists of short paragraphs that offer concise, high-quality, appealing features of the decision-making examination. The text part (explanation) is an overview consisting of basic information on the specific subject. A problem that emerges from such a model, which necessitates brevity of information, is that important features of information are omitted. For example, it would be difficult to diagnose "psychiatric dizziness," as suggested (p 22) on the basis of "psychiatric symptom cluster (e.g., dizziness during a panic attack)." There are a complex of symptoms that make up this syndrome.

Each section (topic) is self-contained on 2 pages that face each other, except for a number of the algorithms that refer to other chapters. The book has a touch of traditional features in the text and a contemporary feature in the format. It is unique. The authors met the goal of the algorithm format: maximizing the amount of information within a minimal amount of text.

Applicability: One of the authors felt that the information in this book would be useful to medical students, residents, and junior practitioners in our specialty. I think, also, that the volume offers a review resource to the more seasoned practitioner.

Dennis G. Pappas, MD
Birmingham, Alabama

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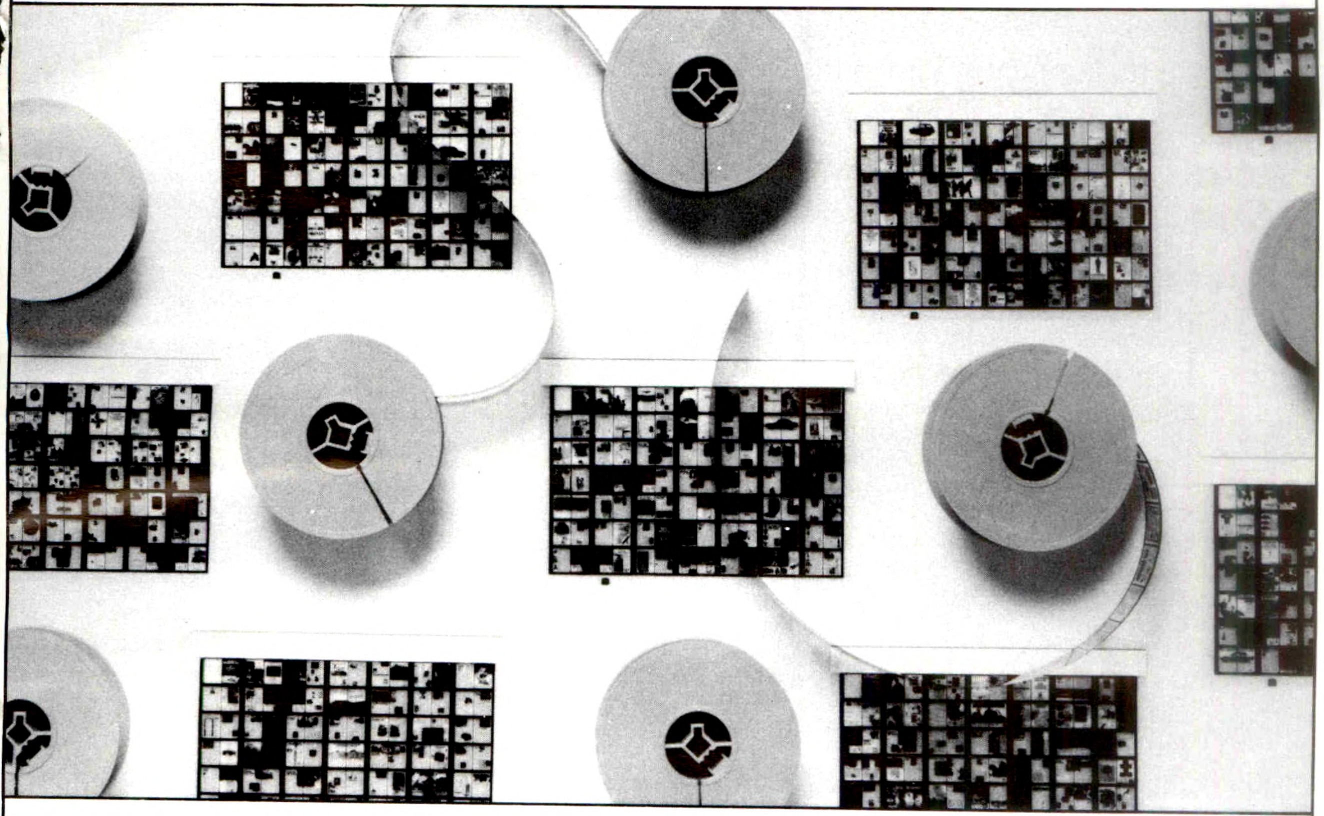

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Available July 1, 2004. The Department of Otology and Laryngology is seeking applicants for a one-year clinical fellowship in laryngology, phonosurgery, and management of the professional voice. The curriculum will provide a wide range of experiences, including phonomicrosurgery (cold instruments and lasers), laryngeal framework surgery, laryngeal electromyography, and the use of botulinum toxin injections for spasmodic dysphonia. The fellow will participate in the management of voice disorders as a member of a multidisciplinary team that includes full exposure to state-of-the-art voice laboratory facilities. The curriculum is designed so that upon completion, the fellow will have sufficient background to establish an independent voice disorders program at his or her home institution. There are numerous clinical and basic science research opportunities available as well as the option to work collaboratively with local music conservatories. Certification by the American Board of Otolaryngology or eligibility is a prerequisite.

Direct inquiries to: Steven M. Zeitels, MD, Director, Division of Laryngology, Massachusetts Eye and Ear Infirmary,
243 Charles Street, Boston, MA 02114;
Phone: (617) 573-3557, Fax: (617) 573-3068

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PEDIATRIC AIRWAY ENDOSCOPY COURSE

UNIVERSITY OF IOWA, IOWA CITY, IOWA

Friday, May 31—Monday, June 3, 2002

This 4-day course, designed for residents, fellows, and practicing otolaryngologists, provides a comprehensive review of the evaluation and management of infants, children, and adolescents with congenital and acquired causes of airway obstruction. Daily didactic sessions will be followed by panel discussions and hands-on animal laboratory sessions demonstrating traditional and novel techniques of direct laryngoscopy and bronchoscopy, CO_2 laser excision of laryngeal and tracheal anomalies, removal of tracheal foreign bodies, and laryngotracheal reconstruction. Intraoperative direct laryngoscopy and bronchoscopy will be observed in patients with pediatric airway anomalies.

Course Directors: Nancy Bauman, MD, FAAP, FACS*
Jose Manaligod, MD*
Richard J. H. Smith, MD, FAAP, FACS*

Assistant Course Directors: Benjamin Cable, MD*
Chantal Giguere, MD*
Stephen Hone, MB FRCSI*

Guest Faculty: Bruce Benjamin, DDO, OBE, FRACS, FAAP
Sydney, Australia
Lauren Holinger, MD, FAAP, FACS
Children's Memorial Hospital, Chicago, Illinois
Andrew Inglis, MD, FAAP, FACS
University of Washington, Seattle, Washington

Registration fee: $1,000
Residents: $750 with letter from departmental head
20 hours of CME credit
Enrollment limited to 25 participants

For further information, contact:

Mary Means
Department of Otolaryngology–Head and Neck Surgery
University of Iowa Hospitals & Clinics
Iowa City, IA 52242
(319) 384-5837

*University of Iowa, Department of Otolaryngology–Head and Neck Surgery

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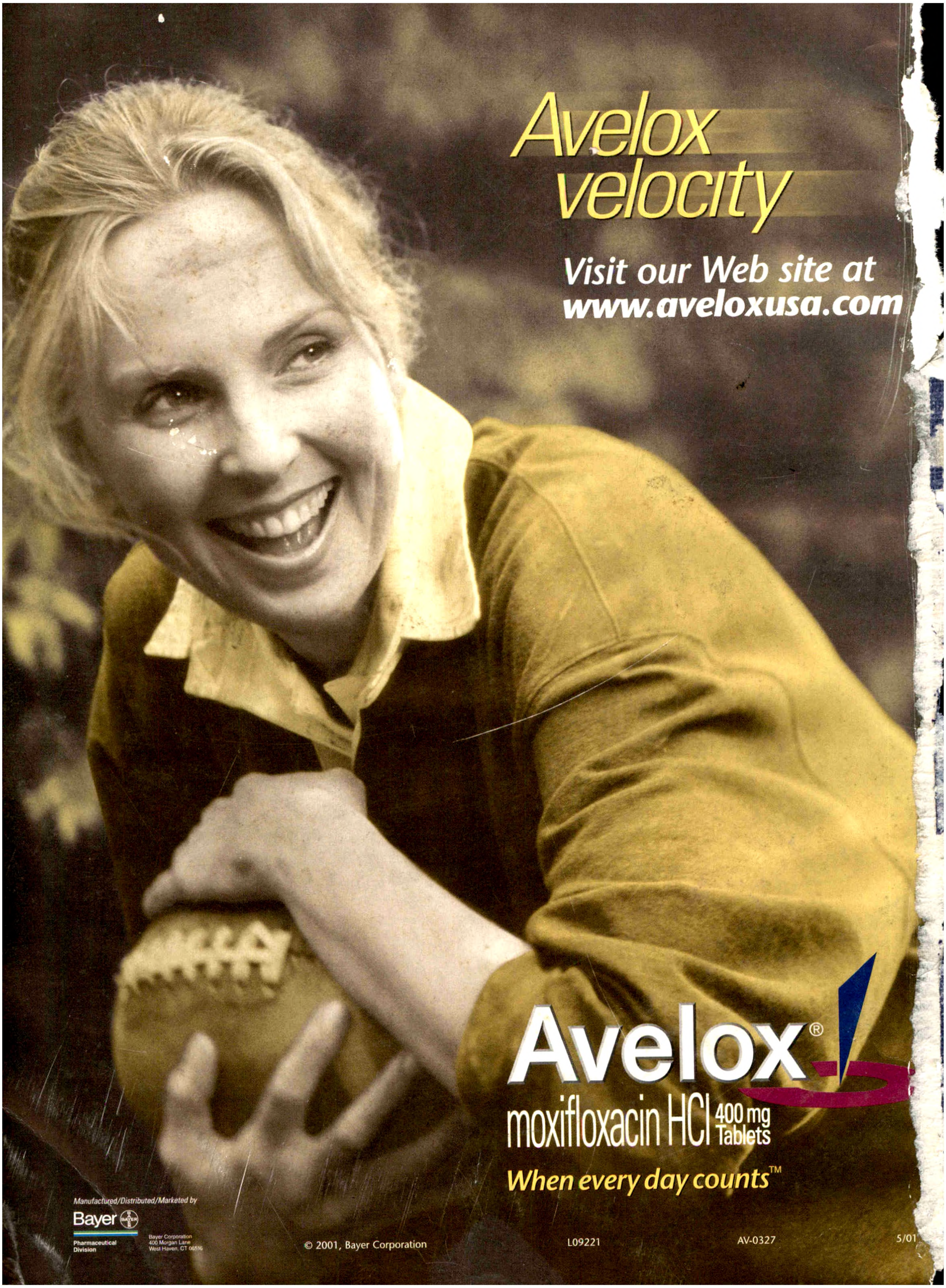
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